Integrative inference of evolutionary patterns of desert biodiversity: a spatial and temporal multi-scale approach using herpetofauna from North-Africa

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Nota prévia

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Resumo

O Norte de África é uma região de grande importância biogeográfica, cada vez mais reconhecida como um centro de biodiversidade e diversificação, e com uma estreita relação histórica com a biodiversidade Europeia. A maior parte da região é ocupada pelo Saara e Sael, duas das principais ecoregiões de África, que abarcam o maior deserto quente do mundo e as regiões áridas vizinhas a sul. A extensão das duas áreas flutuou largamente nos últimos milhões de anos, devido a ciclos de humidade-aridez com 20 000 a 100 000 anos que fizeram o deserto ir e vir, o que, juntamente com uma série de eventos geológicos e uma paisagem topograficamente variada, providenciam um cenário rico para estudar os mecanismos evolutivos que modelam a biodiversidade local.

Pese embora um crescente corpo de estudos acerca da biodiversidade do Saara-Sael, existe ainda um enorme vazio de conhecimento no que toca à distribuição da biodiversidade, aos limites das espécies, ou aos processos evolutivos que levaram aos padrões de biodiversidade presentemente observados. O objectivo geral desta tese foi estender o conhecimento acerca da biodiversidade e história biogeográfica da região árida do Norte de África, assim como dos processos evolutivos que lhe deram forma. Quatro objectivos foram delineados para esse propósito: 1) aumento do volume de dados de amostragem disponíveis para a região, e descrever padrões de diversidade; 2) desenvolvimento e optimização de ferramentas e protocolos orientados para o estudo da biodiversidade local; 3) estudo de padrões evolutivos a grandes escalas espaciais e temporais; e 4) estudo de padrões evolutivos à escala espacial (e temporal) local. Isto foi alcançado integrando ferramentas filogeográficas e eco-geográficas na análise de dados de distribuição, genéticos, e ecológicos referentes a representantes da herpetofauna do Norte de África, incluindo espécies do género *Agama*, *Psammophis schokari*, e *Hoplobatrachus occipitalis*.

A área de distribuição conhecida de várias espécies foi estendida e melhor detalhada, tanto em termos de registos de presença como diversidade genética. A parapatria foi o padrão dominante na distribuição de espécies e linhagens genéticas dos objectos de estudo. Mostrou-se que o clima desempenhou um papel preponderante na formação dos padrões de biodiversidade e evolução na região, influenciando extensões de ocorrência, causando flutuações demográficas e extinções locais, ou restringindo a dispersão, o que em última instância levou a eventos de diversificação e especiação. A hipótese de vicariância induzida pelo clima como principal motor de diversificação foi

testada no género *Agama*, avaliando a ocorrência de padrões biogeográficos esperados e usando comaprações de nichos ecológicos. Os resultados aparentam suportar esta hipótese. Exemplos particulares de agentes vicariantes detectados incluem a cobertura de áreas rochosas devido à deposição de areia (para *A. boulengeri*), ou a bacia do paleo-rio Tamanrasset (para *P. schokari*). A hipótese de que os corredores trans-Saarianos permitem o fluxo génico entre populações de espécies mésicas foi avaliada usando a espécie *P. schokari*. A permanência do corredor mésico do Saara Atlântico foi suportada, pelo menos para espécies mésicas que tendem para o extremo xérico e com alta dispersão como *P. schokari*, possivelmente funcionando até como refúgio. A conectividade entre a costa mediterrânea e o sul da Argélia revelou-se mais evelada do que o esperado. A avaliação biogeográfica à escala local de *A. boulengeri* revelou um cenário complexo, possivelmente envolvendo mútiplos refúgios, efeitos de gargalo recorrentes, connectividade na paisagem variável, e dispersão enviesada a favor dos machos, o que demonstra numa só espécie a riqueza de processos evolutivos que podem intervir na região.

Mecanismos de diversificação não relacionados com o clima também foram identificados na região, com uma possivel alopoliploidização em *H. occipitalis*, que terá isolado uma população endémica nas montanhas da Mauritânia, rodeada por outra tetraplóide largamente difundida. Espera-se que pressões selectivas para adaptação a novas condições também tenham desempenhado algum papel na diversificação das espécies, embora nestes objectos de estudo nenhuma indicação forte de adaptação divergente tenha sido encontrada. Contribuições técnicas foram também feitas com o desenvolvimento de microssatélites para *A. boulengeri* e outras espécies no mesmo género, o que deverá permitir seguir questões respeitantes ao fluxo génico, conectividade na paisagem, ou sistemas de reprodução e dispersão.

Foram providenciados indícios da importância das regiões montanhosas como centros de biodiversidade e refúgios durante os ciclos climáticos do Plioceno-Pleistoceno. O vale de Djouk, que separa as montanhas do Tagant e Assaba na Mauritânia, parece ser de particular interesse para conservação, dado a riqueza de biodiversidade e a importância para a preservação de processos evolutivos e fluxo génico entre sistemas montanhosos vizinhos. Tendo em conta a panóplia de questões endereçadas e a diversidade de áreas relacionadas, eu espero que a contribuição aqui apresentada venha a ser útil para trabalhos futuros em disciplinas variadas, tais como filogeografia, ecologia evolutiva, ou conservação da biodiversidade.

Palavras-chave: ambientes áridos; biogeografia; ciclos climáticos; diversificação; especiação; evolução; filogeografia; modelos de distribuição de espécie; nicho ecológico; Norte de África; Saara; Sael.

Summary

North Africa is a region of high biogeographic importance, increasingly recognized as a biodiversity and diversification hotspot, and with a close historical connection with the European biological diversity. Most of the region is occupied by the Sahara and Sahel, also two of the major ecoregions of Africa, which comprise the world's largest warm desert and the neighbouring arid regions to the south. The range of both areas has widely fluctuated in the last few million years, due to 20-to-100 thousand-years arid-humid cycles that have made the desert come and go, which, together with a series of geological events and a topographically varied landscape, provide a rich scenario to study evolutionary mechanisms shaping the local biodiversity.

In spite of a recently increasing body of studies on Sahara-Sahel biodiversity, there is still a huge knowledge gap regarding biodiversity distribution, species limits, or the evolutionary processes that led to the currently observed patterns of biodiversity. The general aim of this thesis was to increase the knowledge on the biodiversity and biogeographic history of arid North Africa, as well as the evolutionary processes shaping it. Four main goals were delineated for that purpose: 1) increase the available sampling data for the region, and describe biodiversity patterns; 2) development and optimization of tools and protocols targeted at studying local biodiversity; 3) assessment of evolutionary patterns at large spatial and temporal scales; and 4) assessment of evolutionary patterns at local spatial (and temporal) scales. This was achieved by integrating phylogeographic and eco-geographic tools in the analysis of distribution, genetic, and ecological data pertaining representatives of North African herpetofauna, including species of the *Agama* genus, *Psammophis schokari* and *Hoplobatrachus occipitalis*.

The known distribution ranges of several species have been extended and better detailed, both in terms of presence records and genetic diversity. Parapatry was the dominant pattern in the distribution of species and genetic lineages of the study subjects. Climate has been shown to have played a major role in shaping the biodiversity patterns and evolution in the region, by shaping distributional ranges, causing demographic fluctuations and local extinctions, restricting dispersal, which ultimately led to diversification and speciation events. The hypothesis of climate-induced vicariance as lead diversification motor was tested in *Agama* genus, by assessing the occurrence of expected biogeographic patterns and using ecological niche comparisons. Results seem to support this hypothesis. Examples of particular

vicariant agents that were detected were sand deposition that covers rock outcrops (for *A. boulengeri*), or the Tamanrasset paleoriver basin (for *P. schokari*). The hypothesis of trans-Saharan corridors allowing gene flow among populations of mesic species was assessed using *P. schokari*. The permanence of the Atlantic Sahara mesic corridor was supported, at least for the species tending to the xeric and high-dispersal end, like *P. schokari*, possibly working as refugium as well. The connectivity between Mediterranean coast and southern Algerian mountains was higher than expected. The local scale biogeographic assessment of *A. boulengeri* revealed a complex scenario, possibly involving multiple refugia, recurrent bottlenecks, landscape connectivity and male-biased dispersal, demonstrating in one species the richness of evolutionary processes that can intervene in the region.

Non-climate related diversification mechanisms were also identified in the region, with a possible allopolyploidisation in *H. occipitalis*, which isolated in a diploid mountain-endemic population in Mauritania, surrounded by a widespread tetraploid lowland one. Selective pressures towards adaptation to novel conditions are expected to have played a role in species diversification as well, although in the study cases here presented no strong indication of divergent adaptation was found. Technical contributions were also made with the development of microsatellite markers for *A. boulengeri* and other species in the genus, which could allow pursuing questions regarding gene flow, variable landscape connectivity or systems of mating and dispersal.

Evidence is provided for the importance of mountain regions as biodiversity hotspots and refugia during climatic cycles in the Plio-Pleistocene. The Djouk valley, separating the Tagant and Assaba in Mauritania, seems to be of particular interest for conservation given the biodiversity richness and the importance for preserving evolutionary processes and gene-flow among neighbouring mountain systems. Considering the range of questions addressed and the diversity of relatable subjects, I expect the contribution here presented will prove useful for future works on disciplines as varied as phylogeography, evolutionary ecology or biodiversity conservation.

Key words: arid environments; biogeography; climatic cycles; diversification; ecological niche; evolution; North Africa; phylogeography; Sahara; Sahel; speciation; species distribution models.

Contents

List of Tables

List of Figures

List of Abbreviations

16S	16S ribosomal RNA gene
AUC	Area Under the Curve
ANN	Artificial Neural Networks
BCE	Before Common Era
BI	Bayesian Inference
BIC	bayes info criter
bss	bootstrap support
RW	Randon-walk
RRW	Relaxed random-walk
c-mos	oocyte maturation factor MOS
CYTB	cytochrome b
DNA	Deoxyribonucleic acid
ENM	Ecological Niche-based Model
FST	Fixation index FST
GAM	Generalised additive model
GIS	Geographic Information System
GLM	Generalised linear model
GCM	Glabal Circulation Model
GPS	Global Positioning System
HWE	Hardy-Weinberg Equilibrium
IUCN	International Union for the Conservation of Nature
Ka	Kilo annum, 1000 years
LD	Linkage Desiquilibrium
LGM	Last Glacial Maximum
LIG	Last Inter-Glacial
Ma	Mega annum, 1 million years
MAXENT	Maximum Entropy
MBD	Male-biased Dispersal
MCMC	Markov-Chain Monte Carlo
ML	Maximum Likelyhood
mid-Hol	mid-Holocene
mtDNA	mitochondrial DNA
ND4	NADH dehydrogenase subunit 4
NN	Nearest Neighbour
NNI	Nearest Neighbour Index
NN-ratio	Nearest Neighbour ratio
NTF3	Neurotrophin-3
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PNC	Phylogenetic Niche Conservatism
POMC	Proopiomelanocortin
pp	posterior probability
RAG2	Recombination activating gene 2
ROC	receiver-operating characteristics
SD	Standard Deviation
SDM	Species Distribution Model

SE	Standard Error
TRI	std error
tRNAs	Transfer ribonucleic acid
tS-corridor	trans-Saharan (mesic) corridor
TSS	True Skill Statistic

Chapter 1

Introduction

1.1. Biogeography and the study of biological diversity

1.1.1.An historical perspective

Earth is heterogeneous in many aspects, not least in the biological viewpoint. Biological diversity, biodiversity, can be organized in many levels, from the molecular level (e.g. genetic level) to the biosphere. Variability can likewise be found and categorized at many levels, from differences between two DNA strands to different biogeographic realms (Fig. 1.1; Dinerstein et al., 2017; Olson et al., 2001). Patterns of biological diversity have been studied since the classical times, with examples of taxonomic systems found in ancient China (Shennong's Bencaojing Pharmacopeia, c.3000BCE), Greece (Aristotle's works, 4th century BCE), or Rome (Plinius, 77-79, *Naturalis Historia*). However, the study of biodiversity with a geographic framework, Biogeography, and the establishment of this discipline only started to come together in the 18th century. Following the development of modern taxonomy by Linnaeus, and the age of exploration, came the observation that species were not the same everywhere. Starting with Buffon, a series of naturalists (Foster, Willdenow, von Humboldt, Candolle) studied patterns of species richness, showing the relation between species' distributions, climate and geography (Cox et al., 2016). However, the historical component of the distribution of species, why and how they came to be where they are, was still missing. The understanding of the processes came later: the findings about Earth's age, climate and sea level change through time, mountain uplifting, or species extinction provided further explaining processes for biodiversity distribution. A particular cornerstone was Darwin's and Wallace's works on evolution by natural selection. The later was incidentally the main responsible for integrating species distributions and evolution and is commonly credited with the establishment of Biogeography (Brown and Lomolino, 1998). During the first half of the 20th century, numerous

palaeontologists described the origin, dispersal, radiation, and decline of land vertebrates (Brown and Lomolino, 1998). The theory of continental drift also came to be accepted during this period.

Biogeographic research continued integrating more knowledge from ecology, evolutionary biology, geology, and physical geography (Dansereau, 1957), and during the 1950s and 1960s the emphasis in biogeography expanded from an evolutionary and historical perspective to a more ecological one, analysing species interactions. This process culminated in the cornerstone publication of the Theory of Island Biogeography (MacArthur and Wilson, 1967), which introduced immigration and extinction as the main processes responsible for species distributions.

The posterior development of molecular systematics caused the advent of Phylogeography (Avise, 2000; Avise et al., 1987), which addresses biogeography from a population genetics and phylogenetic perspective. Being able to directly observe the inheritable genetic material instead of just phenotypical surrogates has allowed answering more complex questions about the evolutionary processes driving species distributions (Avise, 2000; Hickerson et al., 2010). Advances in computational power in recent years allowed the widespread utilization of Geographical Information Systems (GIS) and ecological niche-based modelling tools, which allowed introducing a spatially explicit component to biogeography.

1.1.2.An integrative multi-scale approach to study biodiversity

The spatial and temporal scales at which researches are able to propose and to test hypotheses have also diversified in recent years. The amount of genetic data that can be easily obtained have massively increased since the invention of polymerase chain reaction (PCR; Mullis and Faloona, 1987), from single-marker short sequences to full genomes in the present. This allows for example analysing gene flow at the genome level or studying processes of ancient hybridization or reticulate evolution (Sousa and Hey, 2013), data that ultimately allow answering more and more complex questions about the evolutionary (and not just the ecological) processes driving species distributions at the local scale. Climatic and land-cover spatial data, as well as past and present climatic models, are available in increasingly higher resolutions and accuracies, to a point where 30cm pixel size data can be commercially obtained from satellite imagery (e.g. DigitalGlobe WorldView-3/4). At the same time, the processes that determine the large-scale distribution of clades can also be addressed through the

integration of phylogenetics and ecology (Pontarp and Wiens, 2016; Wiens, 2012; Wiens and Donoghue, 2004), showing the importance of integrating multiple spatial and time scales when addressing the biogeographic history of a regions or the evolution and distribution of given taxa.

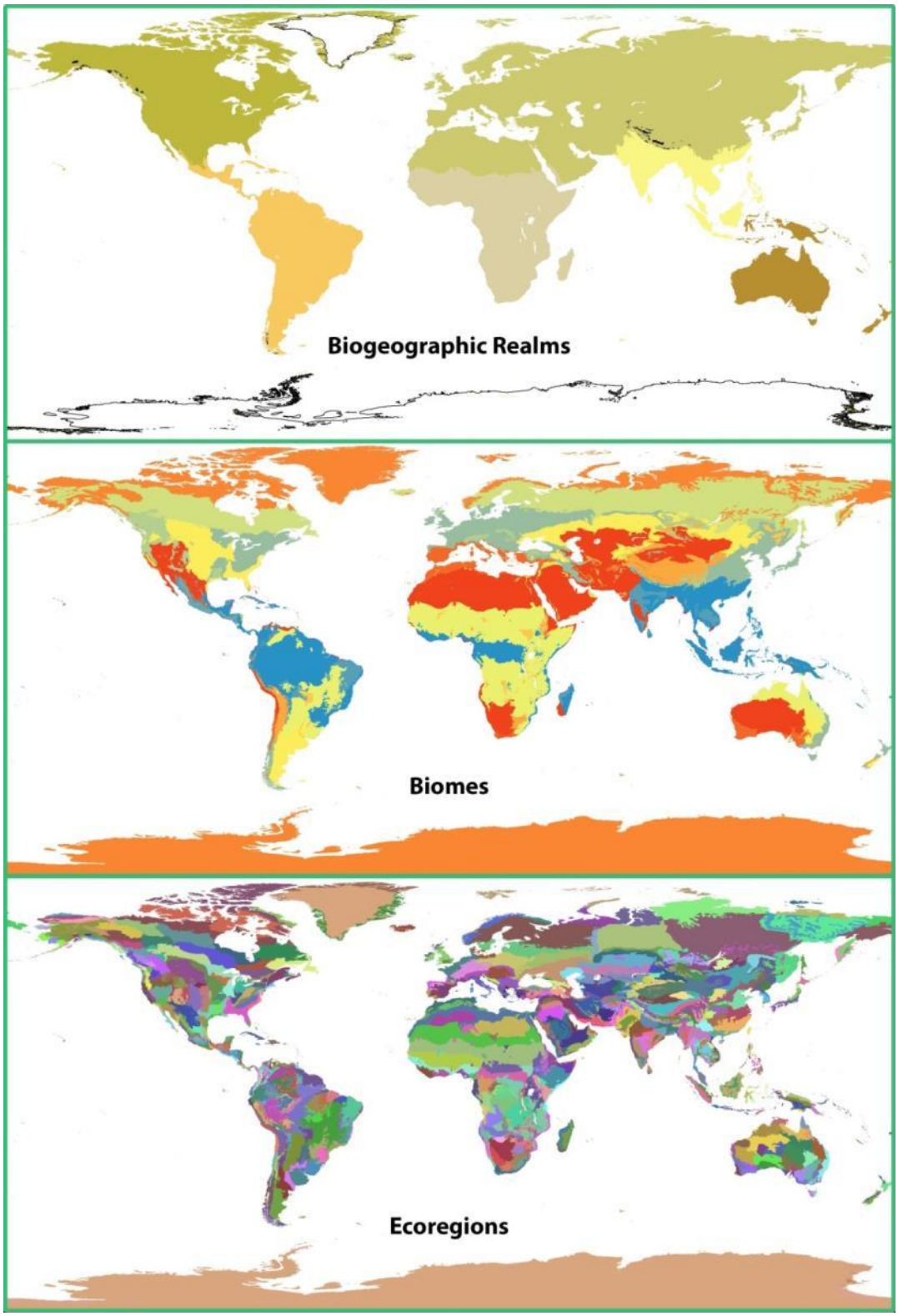

Figure 1. 1. Terrestrial biogeographic regions, representing biogeographic realms, Biomes, and Ecoregions. Adapted from Dinerstein et al. (2017).

1.2. Evolution and diversification of biological diversity

1.2.1.Evolutionary mechanisms

The evolution of biological diversity, the change in the properties of groups of organisms over the course of generations or the descent with modification from a common ancestor (Futuyma, 2005), also works at different scales. For evolution to occur, inter-individual variability is a requirement. Mutation, an alteration of the genome and the ultimately source of genetic variation, occurs at the molecular level, and can go from the alteration of a base to gains and losses of chromosomes. Point mutations, genetic rearrangements (e.g. inversions, duplications, insertions, deletions; Herron and Freeman, 2014), recombination (the mixing of alleles, typically by crossing-over in homologous chromosomes during sexual reproduction; Futuyma, 2005), or other non-mutational mechanisms, like horizontal gene transfer (sharing of genetic material between organisms that are not parent-offspring, most common among prokaryotes – Soucy et al., 2015), alter the genomes of individuals and their descents and therefore the gene-pool and allele frequency of a population. The frequency of alleles in a population can then be mediated at higher organization levels (e.g. specimen or population) by natural selection (including sexual selection), gene flow, and drift.

Natural selection can occur whenever there is a difference in fitness (reproductive success) among individuals due to differences in their phenotype (Losos, 2014). Individuals can be specimens of a species, but also genes, genotypes, cell types of a multicellular organism (e.g. cancer cells), different populations of the same species, or different species. Gene flow is the sharing of alleles between populations, and is usually dependent on individual mobility. Gene flow can bring advantageous alleles into a population, or conversely disrupt the selective pressure towards adaptation to local conditions. Drift accounts for stochastic change in allele frequency, for instance the extinction of a part of population due to a volcanic eruption, the pool of alleles present in a population that was introduced to a given island, or the allele mix in each different sperm. Such drift has greatest effect in small populations and for selectively “neutral” alleles, those that have very small effects on fitness (Losos, 2014).

Obviously, these mechanisms make no sense when considering an organism in isolation. They are mediated by the surrounding environment and influence an individual’s ability to respond to it. Even mutations can be directly affected, as exemplified by environment-induced genomic instability (Galhardo et al., 2007). Environmental factors can be biotic (e.g. competition, trophic relations) or abiotic (e.g.

climate, land-cover, topography). Diversification, and ultimately speciation (or extinction) is what results from the interaction of environmental factors, be it ecological interactions, climatic conditions or geological events, and the previously mentioned evolutionary mechanisms.

1.2.2.Diversification and speciation

In the classic model, speciation has been hypothesized to require some type of isolation of populations, followed by divergence in traits such as morphology, behaviour or habitat use (Herron and Freeman, 2014). A posterior contact of the two divergent populations would be considered as a secondary contact event. While the required isolation was of geographic nature in earlier frameworks, we know now that speciation can occur in sympatry (Bolnick and Fitzpatrick, 2007), or even resulting of gene flow (Arnold, 2015). Therefore, perhaps safe in some cases of geographic isolation, the two steps cannot be considered independently from each other. As an example, something that could be classified as an isolation mechanism, the temporal isolation in the reproductive seasons of two northern ecotypes of the Japanese winter moth, can also be classified as a divergence mechanism (adaptation to different climatic of habitat

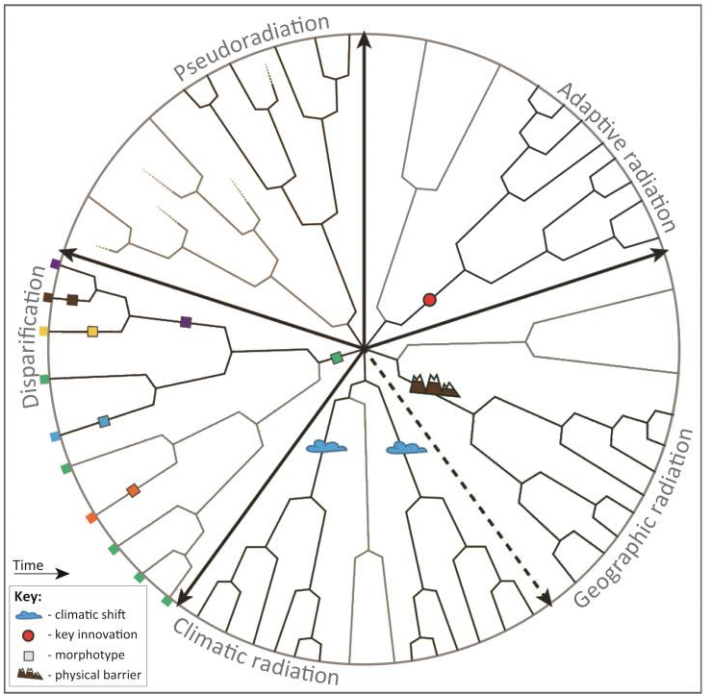

Figure 1. 2. Different types of evolutionary radiations and related phenomena. Radiating clades are represented by black lines. Exaptive radiations (similar to adaptive radiations, but resulting from a new selective regime on an already existing trait, rather than a key innovation) would have a similar pattern to adaptive radiations. Climatic radiation can be considered a type of geographic radiation (separated by broken arrow). Disparification corresponds to a disproportional increase of diversity in morphospace of the “radiating” clade when compared to the “non-radiating” clade. In a pseudoradiation a clade shows higher diversity compared to other owing to a relatively lower in extinction rate; extinct lineages are displayed as broken lines. Adapted from Simões et al. (2016).

conditions), especially since no such distinction exists in the southern populations of the species (Yamamoto and Sota, 2009). Geographic isolation of populations can be due to an event of migration (e.g. island colonization) or vicariance (e.g. the formation of a desert). But with or without allopatry, several mechanisms can lead to divergence and speciation, including for instance divergent selective pressures, or accumulated genetic incompatibility (Losos, 2014).

Divergent selective pressures are probably the most well documented case of speciation process. Adaptation to abiotic factors like climate, or biotic interactions with other species (e.g. predation, parasitism, competition) are probably the most common cases, and can be classified as ecological speciation (Losos, 2014). However, it is also possible that populations subject to the same selective pressures will develop different sets of mutations that will set them apart, what could result in a mutation-order speciation. Sexual selection and assortative matting can also provide the necessary pressure to create a speciation event (Servedio, 2016). Genetic incompatibility can likewise lead to speciation, even in the absence of divergent selection. Drift can result in speciation by gradually causing divergence between to geographically isolated populations; however, punctual changes in the genome can cause reproductive isolation much faster. Polyploidisation (e.g. Brunes et al., 2010) is probably the most obvious instance, but much smaller mutations can have equally radical result, as exemplified by the *Euhadra* snails in which a single-gene controls the direction of the shell coiling and therefore a point mutation can immediately result in two reproductively isolated populations. Speciation can also occur due to hybridization, with hybrid species being maintained through reproductive isolation (genetic, ecological) or selective pressures (Mallet, 2007).

Efforts have been made to organize and categorize types of speciation resulting from the interaction of evolutionary mechanisms and the environment, exemplified by recent classifications of evolutionary radiations that go beyond the adaptive framework (Simões et al., 2016). Concepts like climatic or geographic radiations illustrate the focus on the roles of abiotic factors in the diversification of clades (Fig. 1.2).

1.2.3.Changing dynamics and traceable signatures

The importance of abiotic factors brings a great dynamism to diversification and speciation processes. The geologic and climatic events that punctuated Earth's history resulted in constantly changing selective pressures that should be taken into account

when addressing the evolutionary history of a given taxon. Some of these events are stochastic (e.g. a volcanic eruption), but other are cyclic, like orbital perturbations. According to Milankovitch theory, cyclic variations in the Earth's orbit eccentricity and axis obliquity and precession are responsible for long-term climate fluctuation patterns (Fig. 1.3). Past changes cannot be directly observed, but fortunately leave traceable signatures in the evolutionary history of a species. Morphology, physiology, genetics, ecological niches, among other features, can be analysed in order to infer evolutionary relationships among species and their biogeographic history.

1.3. Inferring evolutionary patterns

1.3.1.Phylogenetics and population genetics

Phylogeography studies the evolutionary footprints that historical processes left on the present geographic distributions of gene-based organismal traits (Avise, 2000). Particularly, it makes use of phylogenetics and population genetics while taking into account the geographical distribution of individuals.

Phylogenetics is the study of evolutionary relationships among individuals or taxa. Methods to compute phylogenies from DNA can treat sequence data as a series of character states, or use them to calculate a distance matrix from which a phylogenetic tree is calculated. They can be further grouped into clustering methods, which cluster data into only one tree, or optimality search criterion methods, which allow comparing several trees (Lemey et al., 2009). Maximum likelihood (ML) and Bayesian inference (BI), both character-state optimality search criterion methods, are probably the most sophisticated and widely used in current phylogenetics (Yang, 2014). ML methods

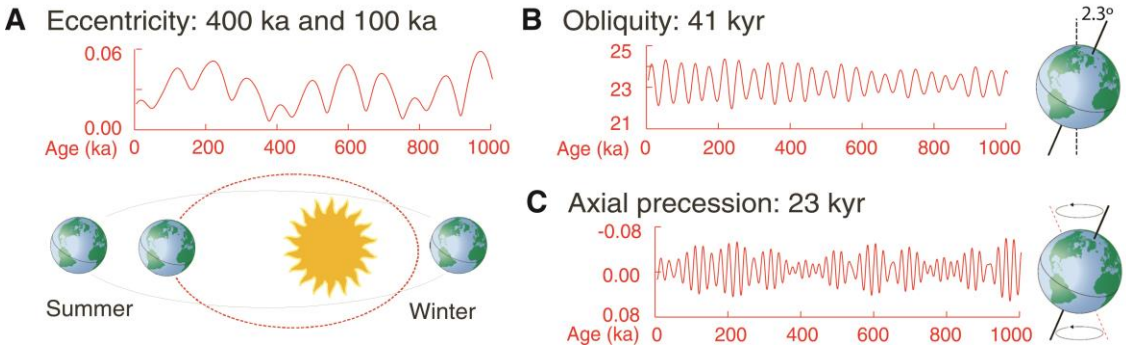

Figure 1. 3. Major orbital perturbations. According to the Milankovitch theory, the affect the long-term climate cycles by changing the amount and reception of radiation reaching the planet. Eccentricity describes the shape of the orbit, and is responsible for the longer-term cycles; Obliquity corresponds to the "wobbling" of the Earth's axis, and affects the radiation reaching the poles; Axial precession corresponds to the orientation of the Earth's axis. Adapted from Zachos et al., (2001).

search for the one tree maximizing the probability of observing the character states, given a tree topology and a model of evolution. Bayesian methods do not search for the single best tree, but rather a group of plausible trees for the data, which allows a confidence estimate of evolutionary relationships. BI requires prior distributions for the model parameters (e.g. substitution model, branch lengths, tree topology), and searches the probabilistic space through Markov chain Monte Carlo (MCMC) sampling technique (Lemey et al., 2009).

Population genetics, as the name implies, deals with the origin and distribution of genetic variation in populations of organisms through space and time (Templeton, 2006). It studies the mechanisms responsible for microevolution (below species level), including adaptation, speciation, or population structure. It therefore overlaps with phylogenetics, although going beyond studying evolutionary relationships.

1.3.2.Species Distribution Models and Ecological Niche-based modelling

Species distribution models (SDM), or ecological niche-based models (ENM), are techniques aimed at extrapolating species distribution data in space and time, usually based on a statistical model (Franklin, 2010). The terminology for these techniques is still a bit confusing, with some authors using different terms, interchangeably and others advocating for terminology in accordance to which niche is actually modelled. Most authors prefer the term SDM, given that the modelling techniques describe empirical correlations between environmental variables and species distributions, and not the actual niche (Franklin, 2010). Others defend that “SDM” should be used to denote only actual distribution models and that “ENM” are potential distribution models based on the niche (Peterson et al., 2008). Regardless, all SDM are based on niche theory (Franklin, 2010).

1.3.2.1. Ecological niche

The first definition of ecological niche referred to the climate and habitat conditions where a species is able to thrive and reproduce (“associational niche” – Grinnell, 1917). Elton, 1927), later defined the niche as the species’ functional role in the biotic community. These definitions evolved into two modern classes of ecological niche: grinnellian niche, defined by non-interactive variables and environmental conditions (e.g. climate), mostly useful at broad scales; and eltonian niche, defined by biotic interactions and resource–consumer dynamics, variables operating mostly at the local scale (Soberón, 2007). More recently Hutchinson, (1957) defined the ecological niche

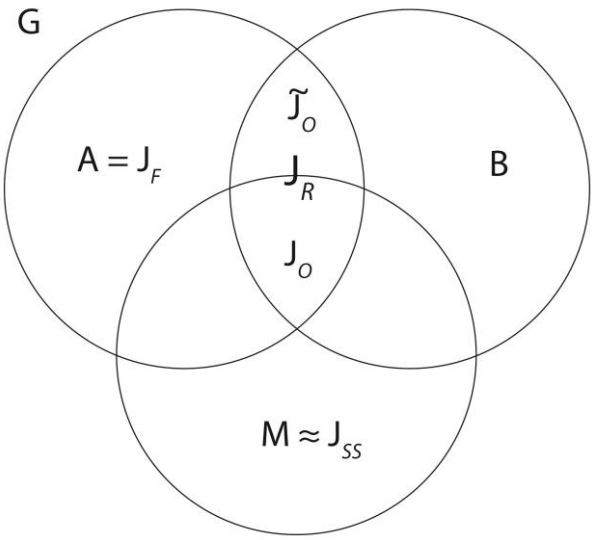

Figure 1. 4. Biotic-Abiotic-Movement (BAM) diagram representing the factors that determine species distributions. **G**: environmental space in the area of study; **A = J_F**: fundamental niche of the species (area with a positive growth rate); **B**: space, defined by biotic interactions, where the species can survive; **M**: area accessible to the species (sinks plus sources **J_{SS}**); **J_R**: occupied area (**J_O**) plus area with the potential to be occupied (**$J\tilde{}_O$**). Adapted from Soberón et al. (2007).

as a hyper-volume of n-dimensions where a species is able to persist indefinitely, and created the concepts of fundamental and realized niche. The first includes the ecological requirements of the species; the second is the part of that multi-dimensional space that the species managed to occupy, and is restricted by variables such as competition or resource availability. The time component to the niche concept was proposed by Pulliam, (2000) and Holt, (2003), with the incorporation of source-sink and dispersal range concepts, respectively. The modern niche concept can be summarised by three components: biotic, abiotic, and movement (Fig. 1.4).

An important trait of ecological niches is a species' tendency to maintain niche requirements similar to that of its ancestors. This has been named Phylogenetic Niche Conservatism (PNC; Harvey and Pagel, 1991). The initial use of the terminology was a bit confusing and was object of a recent review (Pyron et al., 2015), therefore the initial concept would usually refer to modern definitions of "conservatism" and "constraint". PNC is an important principle of ecology and evolution (Peterson, 2011; Pyron et al., 2015; Wiens et al., 2010). It can, for instance be correlated with species-richness patterns (Rosser et al., 2012; Stevens, 2011; Wiens, 2011), or be associated with allopatric speciation by restricting adaptation to novel conditions in a changing environment (Wiens, 2004; Wiens et al., 2009). PNC is also a frequent assumption of ENMs (Elith and Leathwick, 2009; Peterson, 2011): when modelling the occurrence of a species it is assumed all populations have a similar niche; when predicting past distributions another assumption is made on niche stability over time. In fact, it seems

PNC is mostly dependent on the time scale, with niches being highly conserved over short to moderate time spans (up to hundreds of thousands of years; Peterson, 2011). Evidence for PNC is common, but examples of diverging niches are not rare either (Pearman et al., 2008). Whether two species' niches are considered conserved or divergent is also linked with the selected variables and the ecological and geographical scales at work (Pyron et al., 2015), therefore assumptions should be analysed for each particular case when dealing with PNC.

1.3.2.2. Modelling techniques

Given that species distributions, within the limits imposed by historical distribution, are determined by their ecology, it should be theoretically possible to induce the said distributions (either spatial or on a theoretical ecological landscape) based on environmental variables and the species' ecological profiles. Such is the premise behind SDM. Models can be derived following mechanistic approaches (Kearney, 2006), which analyse factors limiting species fitness, sometimes without the need for distribution records (Kearney, 2006; Kearney et al., 2008), and correlative approaches, which relate species distributions with ecological variables at those localities in order extrapolate the probability of occurrence of said species over space (Franklin, 2010). Correlative models are generally favoured for non-models organisms, both given the effort required to determine a species' response to mechanistic variables, and due to the readily available global-coverage climatic layers for past, present, and future conditions (Franklin, 2010). Correlative models can be categorised into presence-only or presence-absence models (Sillero, 2011). Due to the ambiguous nature of recording species absence from a given location (Lobo et al., 2010), artificially generated pseudo-absences are often used (Pearce and Boyce, 2006; Zaniewski et al., 2002), and are often the only viable alternative for remote or scarcely sampled areas.

During the last three decades several modelling techniques have been developed, from simple climate envelope to machine learning (Elith and Leathwick, 2009; Guisan and Thuiller, 2005). A few commonly used techniques are generalized linear models (GLM), generalized additive models (GAM), artificial neural networks (ANN) and Maximum entropy (ME). Linear (regression) models are model-driven approaches aiming at constructing a function that relates the presences and variables (predictors) in order to predict the occurrence (response; Franklin, 2010). However, since all the data is used to estimate a linear relationship, the models are prone to low variance and high bias (Hastie et al., 2001). GLM extend linear models in order to fit non-normal distributions of the response variable (Venables and Ripley, 1994). GAMs are non-parametric

extensions of GLMs (Guisan et al., 2002), modelling non-linear relationships of predictors and response, and differing from GLM in the ability to characterize the response function (Yee and Mitchell, 1991). Machine learning techniques, instead of estimating the parameters for a chosen model, learn the classification rules directly from the training data (Breiman, 2001; Gahegan, 2003). ANN first derive composite variables that are linear combinations of the predictors (inputs), then proceed to model the response (output) as a non-linear function the composite variables. Maximum entropy is a general-purpose machine learning method based on the principle that, within given constraints, a distribution as close as possible to uniform (with maximum entropy) is the best approximation of an unknown distribution, because it agrees with all that is known while avoid assumptions on anything that is not known (Phillips et al., 2006). The later technique is one of the most adequate for presence-only data (Elith et al., 2006).

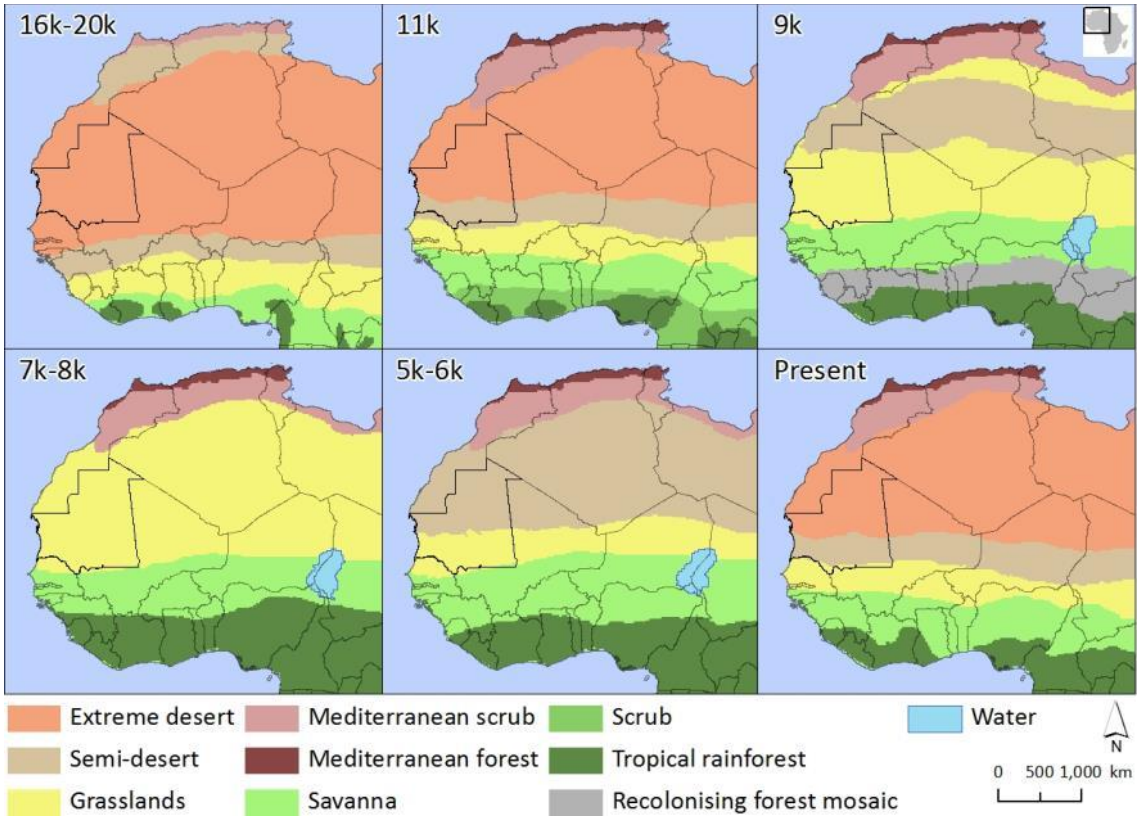

Figure 1. 5. Representation of paleo-vegetation changes since the Last Glacial Maximum (LGM), a likely approximation to the fluctuations during the Pleistocene climatic cycles. Adapted from Adams and Faure, (2004).

1.4. Setting the scenery: biogeographic history of North Africa

Since the Oligocene, North Africa has been affected by a series of geological and climatic events that shaped the current geographic and biological diversity (Fabre, 2005; Le Houérou, 1997, 1992). These included the opening of the Mediterranean to the Atlantic around 7-9 million years ago (Mega-annum, Ma), the subsequent closure 6 Ma and re-opening 5.3 Ma (MSC, Krijgsman et al., 1999), recurrent episodes of desiccation and refilling in the Red Sea area (Bosworth et al., 2005; Girdler, 1991), marine transgressions (Tawadros, 2011), or the uplift of the Atlas mountains. For a revision on revision on Central and Western Mediterranean geology see de Jong, (1998).

Climatically, the most wide-ranging event was a shift from a tropical to an arid environment around mid-Miocene (Zachos et al., 2001) that eventually led to the appearance of the Sahara desert, estimated between 7 Ma (Schuster et al., 2006) and 2.5 Ma (Swezey, 2009). Fluctuations in temperature and precipitation regimes then caused a series of expansions and contractions of climatic zones, leading to a repeated intercalation of arid and humid conditions at intervals of 20-100Ka during the

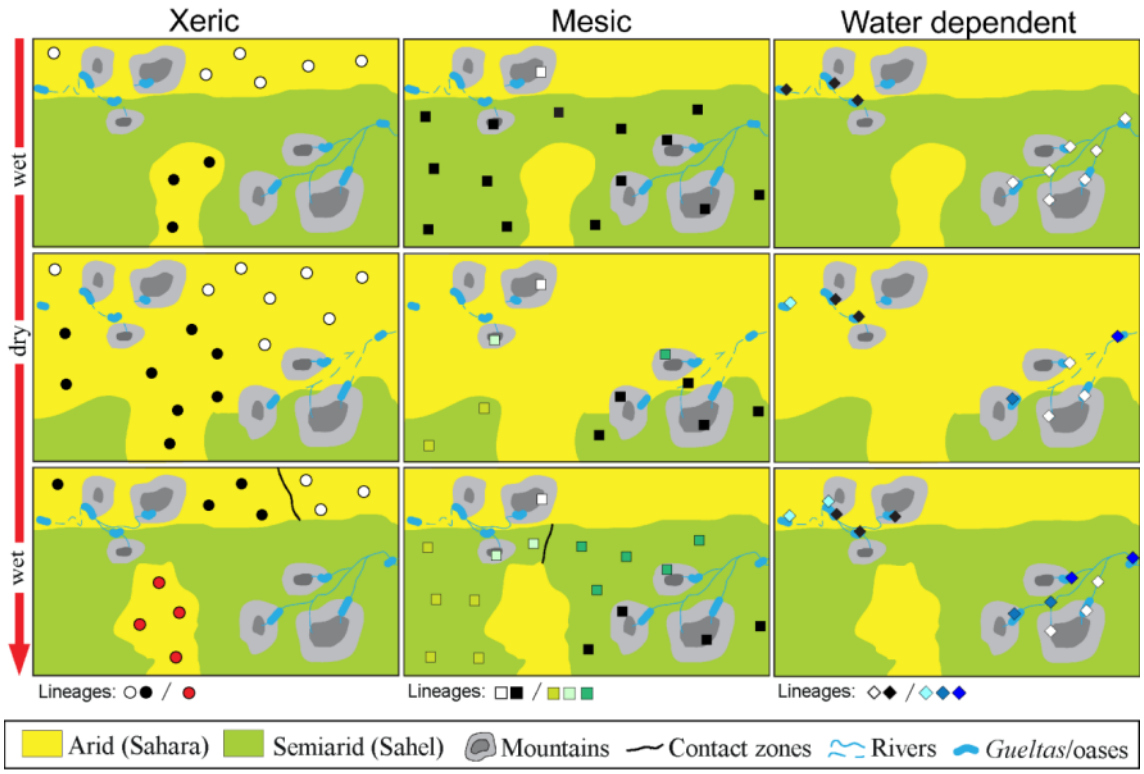

Figure 1. 6. Summary of hypothetical diversification mechanisms through allopatric processes expected for three types of Saharan-adapted species: xeric (circles), mesic (squares) and water-dependent species (diamonds). A time series of climatic cycles is shown from top to bottom. Wet periods lead to expansion of semiarid environments (Sahel), while dry periods lead to wider arid environments (Sahara). Cycles of range expansion-contraction lead to the formation of new lineages (colours) and subsequent contact zones between lineages (black lines).

last few million years (Le Houérou, 1992; Swezey, 2009; Trauth et al., 2009). Debate persists on whether the state switch events were more sudden or gradual, as exemplified by research on the last aridification in mid-Holocene (Holmes, 2008), but given the geographical scale at which they occurred they were still relatively quick (Fig. 1.5).

During humid phases, hyper-arid regions and xeric species' (occurring in habitats with <200mm precipitation) ranges were heavily reduced and probably isolated, while in arid phases the more mesic species (inhabiting areas that are wetter, but never saturated by water) were pushed towards the coastal areas and up the mountains (Le Houérou, 1992; Messerli and Winiger, 1992). This interaction of climatic oscillations and landscape established the conditions for demographic fluctuations, adaptation, range shifts, dispersal, vicariance and secondary contact events that have played a major role in the currently observed patterns of biodiversity (Fig. 1.6; Brito et al., 2014). Climate fluctuations seem to have promoted diversification through several mechanisms, including by creating the conditions for rapid genome re-patterning (Dobigny et al., 2005), ecological adaptation to novel habitats (Boratynski et al., 2012; Carranza et al., 2002; Guillaumet et al., 2008) or, more commonly proposed, divergence in allopatry due to climate-induced vicariance (Brito et al., 2014).

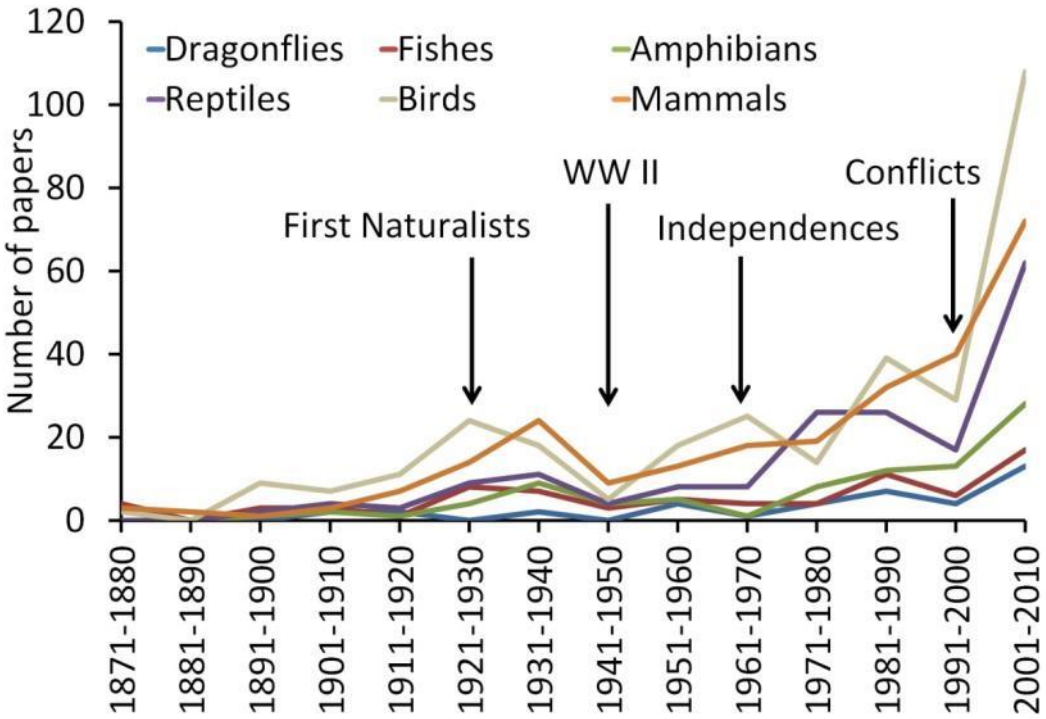

Figure 1. 7. Past publication trends pertaining Sahara-Sahel biodiversity. Six animal groups are separately represented, as well as key historical events in the region. From Brito et al. (2014).

1.5. The Sahara and the Sahel

1.5.1.Characterization of the region

Sahara and the neighbouring regions (Fig. 1.8) have a special place in human history and imaginary. This was the greatest barrier to transpose in our out-of-Africa journeys, it figures in numerous accounts since ancient times, it has spawned a good number of myths and mythological entities, gave rise to peculiar human societies, and still today is seen as an ultimate challenge for human endeavour (Paris-Dakar, Marathon des Sables). The Sahara desert and the neighbouring arid Sahel are also two of the major ecoregions of Africa (Olson et al., 2001), but in spite of all this the amount of research dedicated to it has been, at least until recently, very scarce (Brito et al., 2014; Fig. 1.7). Deserts and arid regions in general are commonly perceived as homogeneous stretches of bare land, with low diversity in comparison to other regions, which probably leads them to attract less scientific attention (Durant et al., 2012). However, these regions present several characteristics of interest both in terms of conservation and evolutionary biology: climatic extremes generating sharp ecological gradients (Schulz et al., 2009), patchily distributed species whose range limits are under strong climatic control, a relatively high rate of endemism due to adaptive processes of organisms to extreme environments, and locally endangered micro-hotspots of biodiversity (Davies et al., 2012; Dumont, 1982; Murphy et al., 2013; Wilson and Pitts, 2012).

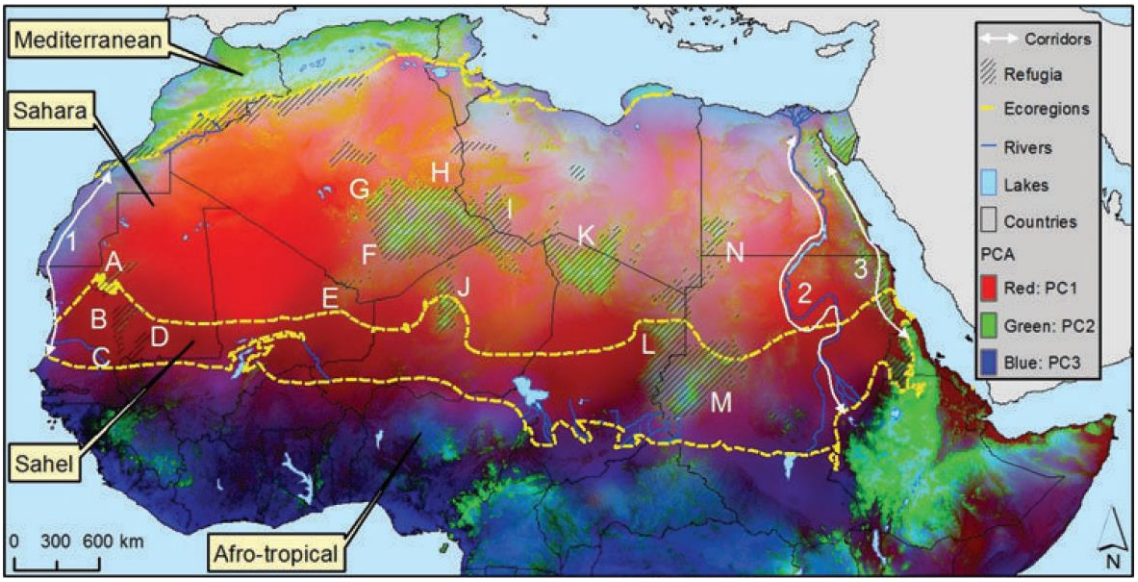

Figure 1. 8. Sahara-Sahel region. The limits of both regions are marked with dashed lines (Olson et al., 2001). Putative trans-Saharan corridors are marked with arrows and numbers: 1, Atlantic Sahara; 2, Nile River valley; 3, Red Sea coast. Capital letters represent hypothesized refugia (mostly mountain systems): A, Adrar Atar-Kediet ej Jill; B, Tagant; C, Assaba; D, Afolle; E, Adrar des Ifoghas; F, Hoggar; G, Mouydir; H, Tassili n'Ajjer; I, Fezzan; J, Aïr; K, Tibesti-Dohone; L, Ennedi-Borkou; M, Marra; N, Uweinat-Gilf Kebir. The colour palate represents the environmental variability and was obtained through a spatial principal components analysis (SPCA): PC1 (44.0%), annual precipitation, precipitation of wettest month, temperature annual range; PC2 (33.4%), altitude, annual mean temperature, minimum temperature of coldest month; PC3 (9.4%): topography roughness index. From Brito et al. (2014).

The Sahara and the Sahel exhibit particular features of interest from a biogeographic perspective: (1) The Sahara is the largest warm desert in the world occupying, together with the Sahel, around 11 230 000 km^2 (larger than Canada). (2) The limit between the Sahara and the Sahel is the transition between the Palaearctic and Afro-Tropical biogeographic realms (Holt et al., 2013; Olson et al., 2001), resulting in increased local biodiversity (Dumont, 1982; Le Houérou, 1992). (3) High topographic variability, from salt pans below sea level (-155m at Lake Assal, Djibouti) to mountains (3415m at Emi Koussi, Chad) constituting a system of "sky islands" (OECD, 2014). (4) Spatially heterogeneous climate, with average annual temperature ranging from 9.4 to 30.8◦C, and average annual precipitation up to 981 mm (www.worldclim.org). In spite of the potential interest, the Sahara-Sahel spreads over ten countries, many rated as low development (UNDP, 2010) and characterised by long-term political instability (OECD, 2014), therefore making field surveys and trans-border research and conservation planning difficult.

A total of seven climatic regions can be identified in the Sahara-Sahel (Figure 1.9; Brito et al., 2016). Climate regions located in the margins of the Sahara-Sahel tend to extend towards the central Sahara through the Atlantic and Red Sea coasts and the Central Sahara Mountains (Fig. 1.9), congruent with a series of putative trans-Saharan corridors/filters that may allow gene flow across the Sahara-Sahel and link refugia for biodiversity (Brito et al., 2014; Coulthard et al., 2013; Drake et al., 2011).

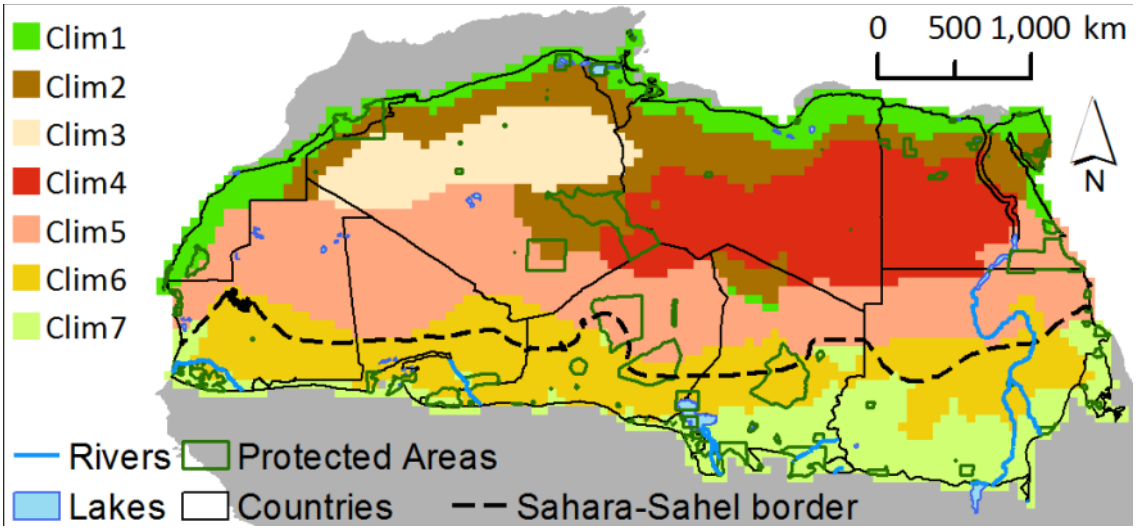

Figure 1. 9. Main climate regions within the Sahara-Sahel, as defined by spatial principal components analyses and model-based clustering algorithm at 0.5 degree resolution (Brito et al., 2016; adapted).

1.5.2.Local biodiversity

Due to the characteristics of the region, data on biodiversity distribution is still severely lacking. Only a few comprehensive and recent distribution atlases are available, mostly biased towards amphibians and reptiles (e.g. Geniez et al., 2004; Trape et al., 2012; Trape and Mané, 2006). However, sampling efforts are being made towards filling the knowledge gap (Brito et al., 2016, 2014). The available data shows that the spatial distribution of biodiversity is heterogeneous, with most species occurring in and around mountain regions and water-bodies (Brito et al., 2014). In spite of the massive geographic scale of the region, micro-refugia seem to be of critical importance for many species (Vale et al., 2015). In absolute numbers, species richness is higher in peripheral, more humid areas, but when considering endemics, central Sahara contains the highest relative richness (Fig. 1.10).

The high rate of endemism reflects the unique adaptations that species had to develop in order to survive the harsh environmental conditions, most notably unpredictable and limited water and food resources, and extreme temperatures and solar radiation. Most desert-dwellers avoid exposure or activity during the hottest parts of the day (midday) and year (dry season). The increased body size of some species reduces the risk of over-heating by increasing thermal inertia, thus allowing activity during the day [e.g. *Psammomys obesus* (Haim et al., 2006)]. Elongated bodies, wedge-shaped head and limb reduction have evolved multiple times in ‘grass swimmers’ and ‘sand burrowers’ [e.g. *Chalcides* spp. and *Sphenops* spp. (Carranza et al., 2008); *Scincus scincus* (Maladen et al., 2009)], adaptations that increase mobility in sand and grass in savannah and desert conditions. Physiological examples include reduced resting metabolic rate and long retention time of fluid in the gastrointestinal tract, connected to reduction of overall energy turnover, as well as lower metabolic rate [e.g. *Addax nasomaculatus* (Hummel et al., 2008); *Lepus capensis* (Kronfeld and Shkolnik, 1996)].

1.5.3.Herpetofauna

Deserts have high herpetofauna diversity. This high diversity seems to be directly related to the arid conditions in some cases, as the skink radiation (Carranza et al., 2008). In others, like the case of phrynosomatid lizards (Wiens et al., 2013) it seems diversity is mostly related to how long a lineage has persisted in a given aridity stratum. Irrespective of the particular mechanism that led to a group’s diversification, amphibians and reptiles display promising traits for the study of evolutionary processes behind vertebrate diversity in the region. In the case of amphibians, their strong

dependence on water availability should allow to closely follow the evolutionary consequences of the fluctuating precipitation regimes. In the case of the reptiles, their ectothermic physiology could make them more responsive to climatic fluctuations than endothermic or volant vertebrates. Also, in general, herpetofauna has likely been less affected by human activities (e.g. in terms of range or population sizes) than for instance mammals or birds (e.g. due to overhunting), thus the present distribution patterns may be more representative of the evolutionary history of taxa.

In order to cover a range of different evolutionary responses to climatic fluctuations, three groups of reptiles and one amphibian may be understood as representatives of evolutionary patterns in the local fauna: 1) *Agama* lizard genus; 2) *Psammophis schokari* snake; and 3) *Hoplobatrachus occipitalis* frog:

1) Part of a family that entered Africa through Arabian Peninsula and likely used arid corridors to colonize the continent (Kissling et al., 2016), *Agama* genus has around 45 species (Leaché et al., 2017) spread throughout Africa. These are common lizards, found in arid and semi-arid habitats that include rocky outcrops, deserts and forests (Le Berre, 1989; Schleich et al., 1996). The genus phylogeny is reasonably well resolved at the species level (Leaché et al., 2014), although the example of recent works on *Agama* suggests further screening at intra-specific level may reveal additional species (e.g. Wagner et al., 2008a, 2008b). Several species occur in the Sahara and Sahel,

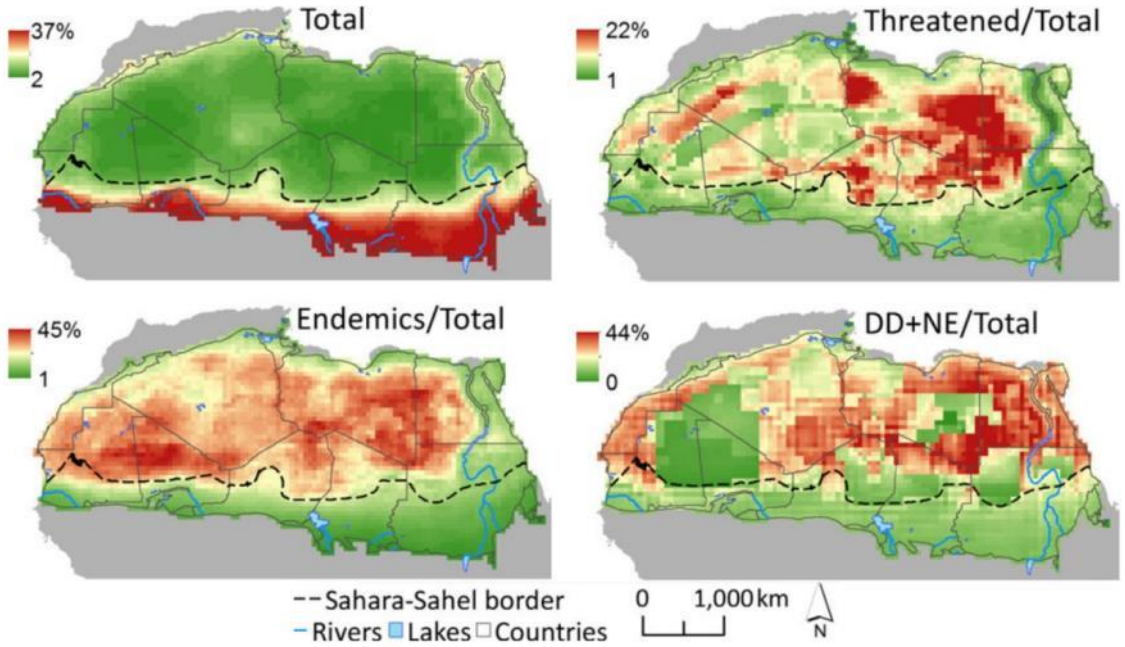

Figure 1. 10. Species richness in the Sahara-Sahel, representing amphibians, reptiles, breeding birds and mammals. The total richness is represented as percentage of a total of 1147 species present in each grid cell (0.5 degree resolution). Richness of endemic species (125 species), threatened species (45 species; categories Vulnerable, Endangered and Critically Endangered) and Data Deficient (DD) plus Not Evaluated (NE) species (121 species) are represented in relation to the total number of species per cell (Brito et al., 2016).

mostly in the western half: *A. boulengeri*, in rocky outcrops in and around the mountains of Mauritania and SW Mali; *A. spinosa* in the mountains along the Red Sea; *A. hartmanni* in Sudan; *A. impalearis* mostly in Morocco; *A. boueti* in the Sahel from Mauritania to Chad and mountains of northern Mali and Niger; and *A. tassiliensis* in the mountains of central Sahara. The *Agama agama* species group, although having most of the distribution south of the Sahel, also occurs in human settlements and arborized areas in the Sahel.

2) *Psammophis* is a genus of diurnal, fast-moving snakes, with 34 species (Uetz and Hošek, 2016) occurring mostly throughout tropical Africa, with some spanning the Middle East and South-Central Asia (Sindaco et al., 2013). Among the most common species in North Africa are *Psammophis schokari* (FORSKAL, 1775), and sister species *Psammophis aegyptius* MARX, 1958 (Kelly et al., 2008; Sindaco et al., 2013). *Psammophis schokari* is a common generalist colubrid occurring from West Africa to India, mostly in desert and xeric scrublands, marginally in sandy habitats in dry Mediterranean zones (Kelly et al., 2008; Schleich et al., 1996; Sindaco et al., 2013). Its sister species *Psammophis aegyptius* is a typical Saharan species and is renowned for inhabiting even the driest and most lifeless areas of the eastern Sahara (Baha El Din, 2006).

3) *Hoplobatrachus occipitalis* belongs to a genus of widespread frogs that originated in Asia. It is the only African representative of this group of tiger frogs, a distribution that resulted from a dispersal event that likely took place in the Miocene (Kosuch et al., 2001). *H. occipitalis* is found throughout Western and Central Africa occurring almost exclusively in permanent water bodies like river banks, rock-pools and savanna ponds, dispersing and reproducing only when rainfall is enough to fill the temporary ponds (Spieler et al., 1997; Spieler & Linsenmair, 1998).

1.6. Academic-niche setting of the present work

In spite of an increasing body of studies on Sahara-Sahel biodiversity, there is still a huge knowledge gap regarding biodiversity distribution, species limits, or the evolutionary processes that led to the currently observed patterns of biodiversity. Sampling is still a major priority for studying biodiversity in the region, particularly in the less accessible areas. Field surveys would improve our knowledge on species distribution, and increase tissue collection for future phylogeographic studies. Integrating phylogenetic inferences with presence records and spatially explicit

ecological layers would provide a more complete picture on the evolutionary processes at play in Sahara-Sahel. Studying the genetic structure of species occurring on both sides of the desert could allow a better understanding of the location of the hypothesized trans-Saharan desert corridors, their permanence and functioning, and role in the diversification of local diversity. Niche models could also be used to understand, through the level of ecological divergence, the relative role of adaptation and vicariance speciation in local fauna. On the other hand, diversification mechanisms at the molecular level would be easier to detect by assessing the genetic profile of populations, since they may not reflect on the distribution or ecological factors. Analysing gene-flow at local scales would allow a better understanding of the processes occurring at more recent time scales, and also locate with certainty contact zones among divergent lineages or create diversity maps that would be invaluable for conservation purposes. However, the availability of optimized fine-scale molecular markers for Sahara-Sahel species is very low, and therefore studies using such approaches are extremely rare. In general, this work tries to improve the foundations for studying the biogeography of the Sahara-Sahel region, while at the same time addressing some of the relevant questions being posed both at the regional and local scale.

The primary source of data for this thesis was field work. Most tissue samples and specimen locations were collected during five expeditions between 2010 and 2014 in which I participated, and then complemented through loans and visits to museum and local collections. DNA was extracted from the tissue samples and resulting data treated in a spatial framework, therefore this work has a strong phylogeographic component. The inclusion of spatially explicit climatic and land-cover information and the use of ecological models conferred the eco-geographical component. These two sets of tools and techniques hold a great potential to inform each other, and major insights could be gained by combining them into an integrative approach.

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Chapter 2

Objectives and thesis structure

2.1. General objectives

The general aim of this thesis was to increase the knowledge on the biodiversity and biogeographic history of arid North Africa, as well as the evolutionary processes shaping it. This is achieved by delineating four main goals: 1) increasing the available sampling data for the region, and describe biodiversity patterns; 2) development and optimization of tools and protocols targeted at studying local biodiversity; 3) assessment of evolutionary patterns at large spatial and temporal scales; and 4) assessment of evolutionary patterns at local spatial (and temporal) scales.

2.2. Detailed objectives and thesis structure

This dissertation is divided in seven chapters. Chapter 1 consists of an introduction to the subjects relevant to the following chapters. I start with an historical perspective on the study of biodiversity, the origin of Biogeography as a scientific discipline, and the increasing complexity and integrative nature is achieved through time. I then introduce the main evolutionary mechanisms, the way they interact with environmental factors, and the resulting processes and patterns of diversification and speciation. The principles and methodological approaches used to infer the evolutionary history of species are then presented with a focus on two main components: phylogeography and niche-based species distribution modelling. The general study area, North Africa, is first presented through a geographic, climatic and biological historical view, followed by a focus on the Sahara and the Sahel. An ecological and geographical characterization of the region is followed by considerations of local biodiversity, particularly herpetofauna and the particular study subjects of this thesis.

In the present chapter 2, I describe the main objectives and particular questions addressed, as well as outline the organization of this dissertation.

In chapter 3, I and my colleagues contribute with new presence data, and comment on the herpetofauna diversity of southern Niger. The observations were catalogued into one manuscript:

- Gonçalves, D.V., Álvares, F., Brito, J.C., 2013. Data on the distribution of herpetofauna of southern Niger with comments on Termit & Tin Toumma National Nature Reserve. Boletín de la Asociación Herpetológica Española 24, 74–78.

While the first objective is addressed throughout most chapters, I isolate this example since it is the most dedicated to said objective. This work was designed with particular attention to the recently classified Termit & Tin Toumma National Nature Reserve, given the marked lack of data available for the region at the time.

Chapter 4 addresses the tool development objective. Here we present a battery of microsatellite markers developed for *Agama boulengeri*:

- Gonçalves, D.V., Pereira, P., Godinho, R., Lopes, S., Velo-Antón, G., Brito, J.C., 2016. Development of 23 microsatellite loci for Boulenger's agama (*Agama boulengeri*) with partial cross-amplification in other *Agama* species. Amphibia-Reptilia 37, 246–252.

Since *Agama boulengeri* occurs in a transition zone between desert and savannah biomes, and most of its distribution is restricted to sky-islands, it was selected as a good candidate for studying contact-zone and gene flow dynamics in the region. The broad application of objective 2 is targeted based on the pan-African distribution of the genus.

Chapter 5 focuses on the evolutionary patterns at large spatial and temporal scales, using amphibians and reptiles as models. This chapter contains three manuscripts:

- Gonçalves, D. V., Martínez-Freiría, F., Crochet, P.-A., Geniez, P., Carranza, S., Brito, J.C., 2018. The role of climatic cycles and trans-Saharan migration corridors in species diversification: biogeography of *Psammophis schokari* group in North Africa. Molecular Phylogenetics and Evolution 118, 64-74.

- Gonçalves, D. V., Pereira, P., Velo-Antón, G., Harris, D.J., Carranza, S., Brito, J.C. Assessing the role of aridity-induced vicariance and ecological divergence in species diversification in North-West Africa using *Agama* lizards. submitted.
- Gonçalves, D.V., Brito, J.C. Phylogeography of *Hoplobatrachus occipitalis* in Mauritania provides another piece to the diploid-tetraploid frog puzzle? in prep.

In the first manuscript we assess the role of corridors in trans-Saharan dispersal, with particular focus on the Atlantic Sahara trans-Sahara corridor, joining phylogeography and palaeoclimatic modelling, and using *Psammophis schokari* as study subject. We addressed two questions: 1) where are the areas with higher climatic stability throughout the species range and particularly West Africa and where are the potential dispersal routes across the Sahara?; 2) how is the genetic variability spatially structured? After analysing the species' genetic structure, we modelled the species' potential distribution in different climatic phases to assess the suitability of candidate trans-Sahara corridors as ecological corridors for *Psammophis*, and by proxy, other mesic species.

The second manuscript uses the concepts of Phylogenetic Niche Conservatism in order to determine the prevalence of aridity-induced vicariance as a motor behind speciation in Sahara-Sahel, by opposition to ecological adaptation. Using species of *Agama* genus as study subject, we tested the occurrence of several patterns expected under vicariant speciation: 1) Prevalent allopatric or parapatric distributions; 2) Allopatric climatic refugia; 3) Niche similarity. In order to do so, we combined phylogenetic inferences, paleoclimatic modelling, and statistical niche comparisons. To analyse the relation of phylogenetic distance and niche distance, we compared niches at three levels: sub-specific, inter-specific and between-genus-branch.

The third manuscript deals with a case whose diversification likely had little influence from the climatic fluctuations in the Pliocene-Pleistocene. Here we analyse the genetic structure of *Hoplobatrachus occipitalis* and provide evidence that two distinct lineages occurring in Mauritania are a product of tetraploidy and not so much vicariance or adaptation mechanisms.

Chapter 6 focuses on the evolutionary patterns at the local scale, using *Agama boulengeri* as a model. This chapter contains one manuscript:

- Gonçalves, D. V., Pereira, P., Velo-Antón, G., Carranza, S., Brito, J.C. Niche conservatism, male-biased dispersal and aridification as drivers for deep lineage allopatry and admixture in a desert reptile (*Agama boulengeri*).

Here we attempt to propose a model for the vicariance end of the vicariance-adaptation dynamic shaping biodiversity and evolution in the Sahara-Sahel. Therefore, we selected a species occurring mostly in sky-islands in the border between the Sahara and the Sahel. Our main aims were to 1) clarify how genetic variability and populations are structured; 2) identify the contact zones among intra-specific groups and determine if gene-flow occurs; and 3) demonstrate the strong relationship between rock and A. boulengeri presence. In order to do so, we employed the fine-scale genetic markers described in chapter 4, and integrated the obtained data with climatic, land-cover and sequence genetic data.

In chapter 7 I discuss the subjects addressed in the previous chapters, emphasizing the general achievements and suggesting questions and hypothesis for future research.

Chapter 3

Data collection

3.1. Article I. Data on the distribution of herpetofauna of southern Niger with comments on Termit & Tin Toumma National Nature Reserve

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Resumen: Se aportan los resultados herpetológicos de una expedición científica en abril-mayo de 2012 al sur de Nigeria, una remota zona que incluye la Reserva Natural de Termit & Tin Toumma. Se observaron 27 especies de reptiles y tres de anfibios. Debido al elevado desconocimiento herpetológico de la región, muchas de las nuevas citas aumentan o cubren grandes vacios en la distribución de las especies halladas.

Keywords: Sahara, Sahel, mountains, overland expedition, dry season.

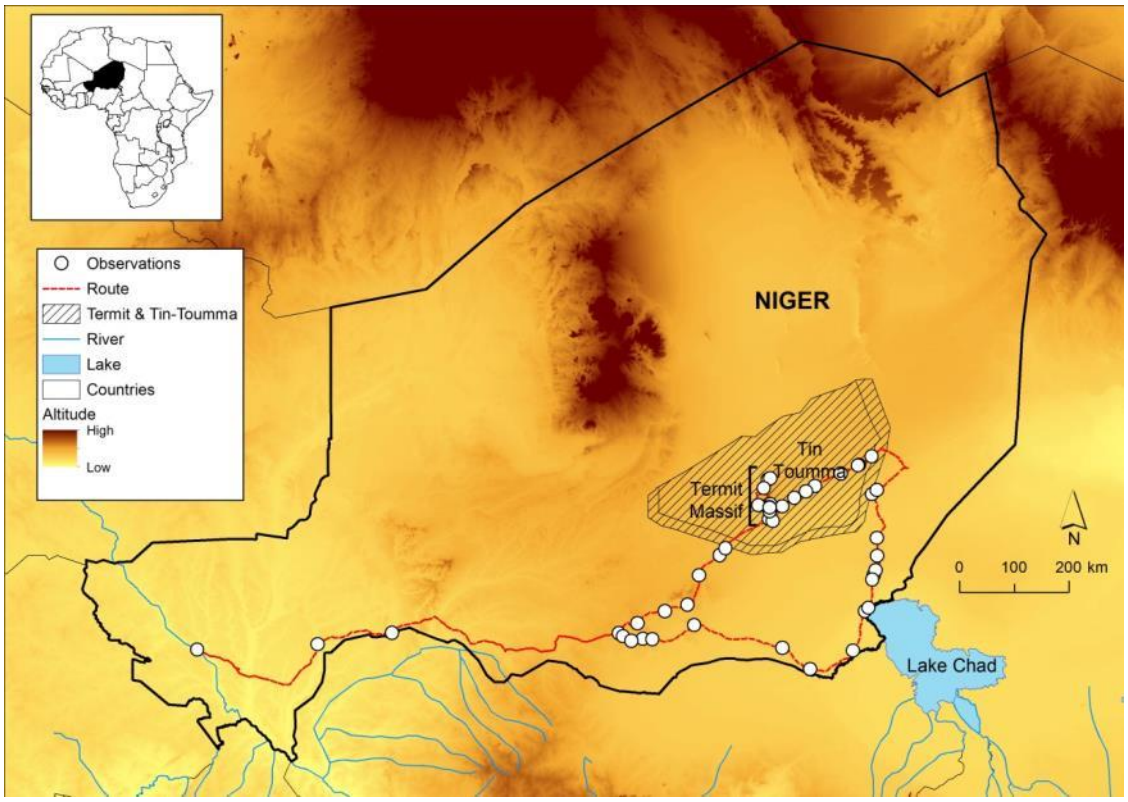

Figure 3. 1. Transect (line) and geographic locations (white dots) of collected observations.

North Africa presents a wide variety of landscapes and habitats which harbours many amphibian and reptile species (Schleich *et al.,* 1996; Chippaux, 2001; Trape and Mané, 2006; Sindaco and Jeremcenko, 2008; Trape *et al.,* 2012). The degree of knowledge varies considerably depending on the region, however. While some areas like Morocco and Western Sahara, or the region near Egypt and the Red Sea are better explored, with atlases available on the distribution of herpetofauna (Baha El Din, 1996; Bons and Geniez, 1996; Geniez *et al.,* 2004), general remoteness is responsible for large exploration gaps. Added socio-political instability and occasional armed conflicts render some areas, like the central Saharan mountains, particularly difficult to access. Niger is one of such cases. Distribution data for the country can be found in classical references (e.g. Angel and Lhote, 1928; De Witte, 1930; Angel, 1950; Guibé, 1950), naturalist work in the second half of the 20th Century (e.g. Heu, 1962; Papenfuss, 1969; Joger, 1981; Kriska, 2001) and broad-scale field guides (Trape and Mané, 2006; Sindaco and Jeremcenko, 2008; Trape *et al.,* 2012). Recently, several works have been published which started to include molecular data (e.g. Crochet *et al.*, 2003; Harris *et al.*, 2007; Rato *et al.*, 2007; Froufe *et al.*, 2009; Fujita and Papenfuss, 2011; Geniez *et al.*, 2011; Gonçalves *et al.*, 2012; Mediannikov *et al.*, 2012). Still, the geographic distribution of many species is poorly known. Here we report our recent

observations of amphibians and reptiles during an expedition to Southern Niger, and compare them with published data. Special emphasis is given to the Termit & Tin Toumma National Nature Reserve, created on March 6, 2012 and totalling almost 100,000 Km^2. This Reserve was primarily aimed at the protection of addax (*Addax nasomaculatus*), dama gazelle (*Gazella dama*), Barbary sheep (*Ammotragus lervia*) and Northwest African cheetah (*Acinonyx jubatus hecki*), as well as supporting the local nomadic people (www.saharaconservation.org/?Termit-Tin-Toumma-Niger), and encompasses the Termit Massif, as well as a vast portion of Tin Toumma desert and part of the Great Bilma Erg (www.ass-niger.org/-termit-tin-toumma-). Twelve reptile species are reported for the approximate area of the Reserve (Trape and Mané, 2006; Trape *et al.*, 2012). Additionally, an online checklist produced by ASS-Niger (Anonymous, 2012) refers 17 reptile species. All species reported to occur have widespread distributions, and both relict populations (e.g. *Agama boueti*, *Latastia longicaudata*) and Sahara endemic species (e.g. *Acanthodactylus longipes*) can be found.

The study area covered Southern Niger and the overland expedition spread over 15 days, between April, 28 and May, 13 of 2012 (Figure 3.1). The sampling strategy involved a transect covering various Sahara and Sahel ecosystems and habitats such as sand deserts (*erg*), firm ground deserts (*reg*), rocky plateaus (*hammada*), dry river beds (*oued*), semi-arid grasslands, savannas, steppes, and thorn shrublands (Figure 3.2). Sampling points were selected along transect based on the diversity of biotopes. A total of 102 observations for 27 reptile species and seven observations for three amphibian species were recorded (see Supplementary Material). Identification of the species was based on published taxonomic keys (Geniez *et al*., 2004; Trape and Mané, 2006; Trape *et al.*, 2012) and posteriorly reviewed by Philippe Geniez (EPHE-UMR 5175) and Pierre-André Crochet (CNRS-UMR 5175) based on photographs. Observations with available photographic data were uploaded to Observado.org (Supplementary Material). It should be noted, however, that due to the website's restriction regarding species names, some records may appear identified differently from this work. For each captured specimen photographs were taken prior to release. The spatial location of specimens was georeferenced by a Global Positioning System (GPS), using the WGS84 coordinate system, and downloaded into a database with an interface for a Geographical Information System (GIS). Comments are made for occurrences expanding distribution limits, taking Trape and Mané (2006) and Trape *et al.* (2012) as reference, or adding information to the knowledge of the natural history of a species (marked with * in Supplementary Material).

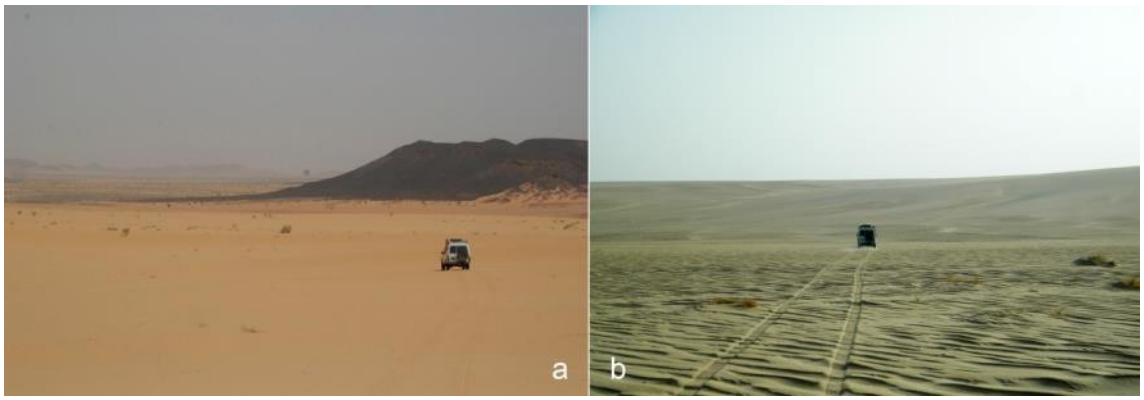

Figure 3. 2. Typical habitats at Termit Mountain (a) and Ti-N-Toumma sandy desert (b).

Reptiles

Agama agama Linnaeus, 1758 – Zinder, Gouré, Mainé-Soroa and Diffa. Found mostly on human settlements. Although the species complex is referenced for Niger and Chad, these records fill a gap of hundreds of kilometres.

Agama boueti Chabanaud, 1917 (Figure 3.3) – Diffa and Kélakam. These specimens were found in the characteristic shrubland habitat, filling a distribution gap in Niger.

Agama paragama Grandison, 1968 – Diffa and Nguigmi. These records add to the northern distribution of the species.

Chamaeleo africanus Laurenti, 1768 – Gouré and Nguigmi. Distribution slightly extended to the East within Niger.

Tropiocolotes steudneri (Peters, 1869) - Diora Aouoranga, Termit. This is the first record for the Reserve, expanding the known distribution more than 250 km to the East, since in Niger it was only known from Aïr Mountains (Trape et al., 2012).

Acanthodactylus cf. senegalensis – Nguigmi and Ngourti. Known species distribution is expanded to the South and East, at about 50 km from Lake Chad.

Acanthodactylus longipes Bouelnger 1921 – Ngourti, Koussa Arma and Ti-N-Toumma. These records expand the distribution in Niger to the East. A huge gap still persists between these observations and the published occurrence in Chad.

Mesalina pasteuri (Bons, 1960) (Figure 3.3) – Ti-N-Toumma. First report for the Reserve and the third record for Niger, although not significantly adding to the species' known distribution.

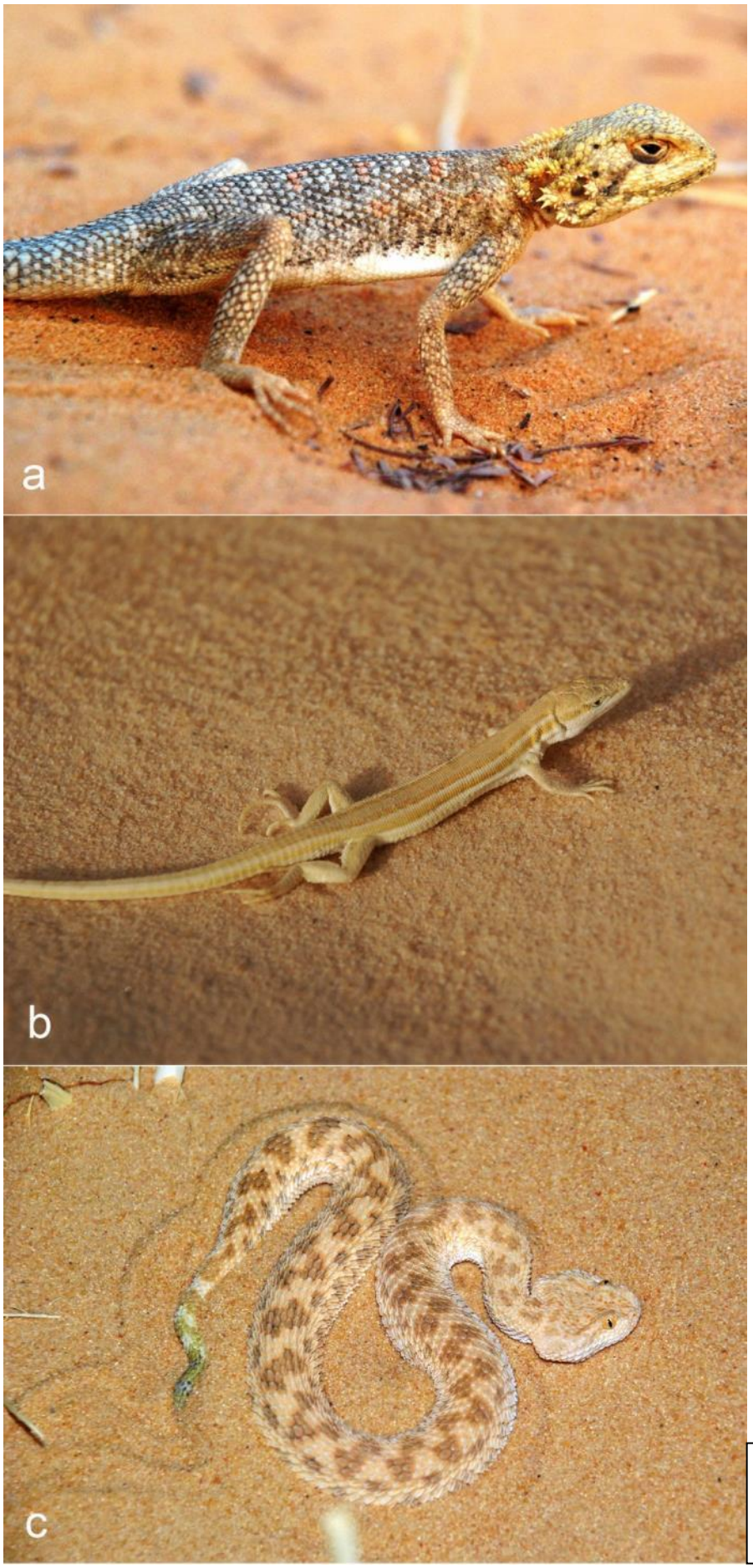

Figure 3. 3. (a) Agama boueti, Termit (observation 6727); (b) Mesalina pasteuri, Tin Toumma (observation 6692); (c) Cerastes vipera, Nguigmi (observation 6658).

Scincopus fasciatus – Kélakam and Tasker. The first point adds to the southern distribution of the species, whereas the second falls in the centre of a previous large gap in Niger.

Scincopus fasciatus / *Scincus albifasciatus* – Madaranga, Termit and Louli Agadem Nga, Termit. Identification of these two records was based solely on tracks. Although it is not possible to distinguish among the species based on the tracks, they are proof that at least one of them is present in the Reserve. Both species are reported for Niger, but assessing their presence in the Reserve is dependent on future surveys.

Pelomedusa subrufa olivacea Schweigger, 1812 – Zinder. This record adds to the area of occurrence in the Sahel. This terrapin is also present in Aïr Mountains (Trape et al., 2012), but there is no information whether there is a suitable habitat connection to Sahelian populations.

Varanus griseus – Ngourti and Ti-N-Toumma. Slightly expanding known distribution in Niger to the East, sightings were far more frequent in Ti-N-Toumma.

Cerastes vipera (Linné, 1758) (Figure 3.3) – Nguigmi. Known distribution in Niger is expanded to the South and East, to less than 50Km from Chad.

During the expedition we registered two confirmed new species, *Mesalina pasteuri*, *Tropiocolotes steudneri*, for the Termit & Tin Toumma National Nature Reserve. Additionally, one other new species for the area is highly likely, *Scincopus fasciatus* or *Scincus albifasciatus*, but still need confirmation (see above). For a total of 18 species reported for the Reserve, we observed eight inside reserve limits plus three more in the near premises. We include *A. boueti* in the confirmed species, given that the referenced *A. impalearis* (online list) is a clear misidentification. The latter species is restricted to the western Maghreb (Trape *et al.*, 2012). The other species not detected in our survey but known from the Reserve (Anonymous, 2012) are: *Eryx muelleri* (Synonym: *Gongylophis muelleri*), *Hemidactylus brooki*, *Psammophis schokari*, *Spalerosophis diadema*, *Tropiocolotes tripolitanus*, *Stenodactylus petrii*, and *Latastia longicaudata*. The latter two are concordant with published data (Trapé *et al.,* 2012). According to Trape and Mané (2006), *Echis ocellatus* also occurs in the Reserve, although it was neither observed by us nor listed online.

The main reason that could explain the lack of observation of several species is the fact that the expedition was performed during the peak of the dry season, when most animals are less active (Cowles and Bogert, 1944). One illustrative example of the unfavourable conditions for animal activity is that the only active *Acanthodactylus* found

at Ti-N-Toumma were juveniles, and were only observed until 11am. This is totally opposite to their behaviour in autumn, when they are active during 10am-16pm (Duvdevani and Borut, 1974; Pérez Mellado, 1992).

All of the species present in the Reserve have widespread distributions across the Sahara and/or the Sahel, and most of them are also reported for major mountain systems in Sahara. Actually, most of the reported species are present in Aïr. Such observations raise the possibility that other species may have also been able to survive until the present around the Termit Massif. Confirmation of this hypothesis requires however additional survey efforts.

This note provides additional data to the poorly known distributions of amphibians and reptiles in Niger and is at the same time the first published list of herpetofauna species present in the Termit & Tin Toumma National Nature Reserve. This constitutes however just another step for the comprehensive mapping of biodiversity in the area and further investigation should ensue.

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Table 3. 1. List of observed specimens, organized alphabetically by Class and Family, with geographic coordinates (WGS84 datum), locality and state when found. Observations with comments marked with *. Observado.org registries are accessible by typing the URL "http://observado.org/waarneming/view/" followed by the individual code. Observations 6653 and 6730 were confirmed by DNA sequencing.

Species	Code	Latitude	Longitude	Local	Province	State	Observado.org code
Amphibians							
Amietophrynus regularis	6606	13.528658	2.052208	Niamey	Niamey	Live	74285181
	6607	13.528658	2.052208	Niamey	Niamey	Live	74285227
Amietophrynus xeros	6617	13.803345	8.978597	Zinder	Zinder	Live	74285542
	6618	13.803345	8.978597	Zinder	Zinder	Live	74285556
Hoplobatrachus occipitalis	6631	13.699577	9.530377	Guidimoni	Zinder	Live	74285596
	6784	13.966488	9.280777	Zinder, 50km NE of	Zinder	Live	74285603
	6785	13.966488	9.280777	Zinder, 50km NE of	Zinder	Live	74285606
Reptiles							
Agama agama	6609	13.528658	2.052208	Niamey	Niamey	Live	74285612
	6611	13.805328	5.253307	Birni N'Konni	Tahoua	Live	74285616
	6613*	13.803345	8.978597	Zinder	Zinder	Live	74285620
	6614*	13.803345	8.978597	Zinder	Zinder	Live	74285623
	6789*	13.966488	9.280777	Zinder, 50km NE of	Zinder	Live	N/A
	6792	13.617213	4.030712	Dogondoutchi, 1km S of	Dosso	Found dead	74285636
	6634*	13.934407	10.21439	Gouré, 8km SW of	Zinder	Live	N/A
	6641*	13.207687	12.117602	Mainé-Soroa, 10km E of	Diffa	Live	N/A
	6650	13.511182	12.823018	Diffa, 30km NE of	Diffa	Live	N/A
	6652	13.511182	12.823018	Diffa, 30km NE of	Diffa	Live	74285659
Agama boueti	6625	13.707633	9.373178	Foret Classe de Kisgoboma	Zinder	Live	74285674
	6638*	13.562232	11.661053	Kélakam, 10km W of	Diffa	Live	74285681

	6649*	13.511182	12.823018	Diffa, 30km NE of	Diffa	Found dead	74285684
	6727	15.867435	11.454855	Termit, Madaranga	Zinder	Live	74285692
	6771	14.748443	10.293282	Kellé, 60km N of	Zinder	Live	74285699
	6775	14.270575	10.103818	Kellé, 5km S of	Zinder	Live	74285707
	6778	14.161528	9.734708	Moha, 5km W of	Zinder	Live	74285714
	6779	14.161528	9.734708	Moha, 5km W of	Zinder	Live	74285716
Agama paragama	6626	13.707633	9.373178	Foret Classe de Kisgoboma	Zinder	Live	74285726
	6648*	13.511182	12.823018	Diffa, 30km NE of	Diffa	Live	74285733
	6654*	14.16534	13.02025	Nguigmi, 15km S of	Diffa	Live	74285738
	6655*	14.16534	13.02025	Nguigmi, 15km S of	Diffa	Live	74285743
	6774	14.270575	10.103818	Kellé, 5km S of	Zinder	Live	74285751
Chamaeleo africanus	6633*	13.934407	10.21439	Gouré, 8km SW of	Zinder	Found dead	74285769
	6656*	14.218497	13.076643	Nguigmi, 3km S of	Diffa	Live	74285778
Psammophis sibilans	6782	14.161528	9.734708	Moha, 5km W of	Zinder	Live	74285792
Crocodylus suchus	6632	13.699577	9.530377	Guidimoni	Zinder	Inquiry	N/A
Hemidactylus angulatus	6605	13.528658	2.052208	Niamey	Niamey	Live	74294174
	6620	13.803345	8.978597	Zinder	Zinder	Live	N/A
	6780	14.161528	9.734708	Moha, 5km W of	Zinder	Live	74294188
Ptyodactylus ragazzi	6608	13.528658	2.052208	Niamey	Niamey	Live	74286627
	6612	13.803345	8.978597	Zinder	Zinder	Live	74286637
	6615	13.803345	8.978597	Zinder	Zinder	Live	74286644
	6616	13.803345	8.978597	Zinder	Zinder	Live	N/A
Tarentola ephippiata hoggarensis	6642	13.207687	12.117602	Mainé-Soroa, 10km E of	Diffa	Live	74294191
	6666	15.366028	13.216635	Ngourti, 5km N of	Diffa	Live	N/A
	6740	15.918648	11.464263	Termit, Zgaidinga	Zinder	Live	74294195
	6753	16.181423	11.356395	Termit, Louli Agadem Nga	Zinder	Live	74294197

	6763	15.670917	11.441985	Termit-Kaoboul, 10km NW of	Zinder	Live	74294200
	6765	15.196902	10.727067	Tasker, 8km N of	Zinder	Live	74294201
	6773	14.270575	10.103818	Kellé, 5km S of	Zinder	Live	74294202
Tropiocolotes steudneri	6716*	15.80434	11.452587	Termit, Diora Aouoranga	Zinder	Live	74294205
Acanthodactylus boskianus nigeriensis	6624	13.707633	9.373178	Foret Classe de Kisgoboma	Zinder	Live	74294211
Acanthodactylus cf. senegalensis	6660*	14.75282	13.163435	Nguigmi, 60km N of	Diffa	Live	N/A
	6661*	14.75282	13.163435	Nguigmi, 60km N of	Diffa	Live	74294219
	6667*	16.0809	13.1347	Ngourti, 80km N of	Diffa	Skin shed	N/A
	6725	15.867435	11.454855	Termit, Madaranga	Zinder	Live	74294227
	6726	15.867435	11.454855	Termit, Madaranga	Zinder	Live	74294231
	6747	16.343802	11.463573	Termit, Nourou Nga	Zinder	Live	74294240
	6764	15.196902	10.727067	Tasker, 8km N of	Zinder	Live	74294245
	6769	15.081673	10.632372	Tasker, 8km S of	Zinder	Live	74294249
Acanthodactylus longipes	6663*	15.071482	13.219652	Ngourti, 30km S of	Diffa	Live	74294252
	6664*	15.071482	13.219652	Ngourti, 30km S of	Diffa	Live	74294254
	6669*	16.149493	13.21645	Koussa Arma well	Diffa	Live	N/A
	6680*	16.704352	13.128903	Ti-N-Toumma	Diffa	Live	74294256
	6681*	16.704352	13.128903	Ti-N-Toumma	Diffa	Live	74294257
	6687*	16.564845	12.928235	Ti-N-Toumma	Diffa	Live	74294264
	6690*	16.423758	12.621273	Ti-N-Toumma	Diffa	Live	N/A
	6696	16.023922	11.860513	Ti-N-Toumma	Zinder	Live	74294276
	6702	15.881558	11.663682	Ti-N-Toumma	Zinder	Live	74294279
Latastia longicauda	6788	13.966488	9.280777	Zinder, 50km NE of	Zinder	Live	74294283
Mesalina pasteuri	6692*	16.217758	12.198542	Ti-N-Toumma	Diffa	Live	74294290
Rhagerhis moilensis	6730	15.867435	11.454855	Termit, Madaranga	Zinder	Skin shed	N/A
Pelomedusa subrufa	6621*	13.749253	9.045058	Zinder, 10km SW of	Zinder	Live	74294295

Scincopus fasciatus	6639*	13.562232	11.661053	Kélakam, 10km W of	Diffa	Found dead	74294370
	6768*	15.081673	10.632372	Tasker, 8km S of	Zinder	Found dead	74294374
Scincopus fasciatus / Scincus albifasciatus	6736*	15.872192	11.446412	Termit, Madaranga	Zinder	Tracks	N/A
	6755	16.181423	11.356395	Termit, Louli Agadem Nga	Zinder	Tracks	N/A
Trachylepis quinquetaeniata	6603	13.528658	2.052208	Niamey	Niamey	Live	74294937
	6604	13.528658	2.052208	Niamey	Niamey	Live	N/A
	6619	13.803345	8.978597	Zinder	Zinder	Live	74294943
	6627	13.707633	9.373178	Foret Classe de Kisgoboma	Zinder	Live	N/A
	6628	13.699577	9.530377	Guidimoni	Zinder	Live	N/A
	6777	14.161528	9.734708	Moha, 5km W of	Zinder	Live	74294952
	6790	13.966488	9.280777	Zinder, 50km NE of	Zinder	Live	N/A
Centrochelys sulcata	6715	15.80434	11.452587	Termit, Diora Aouoranga	Zinder	Live	74295070
	6721	15.80434	11.452587	Termit, Diora Aouoranga	Zinder	Tracks	N/A
	6751	16.343802	11.463573	Termit, Nourou Nga	Zinder	Live	N/A
Varanus exanthematicus	6783	13.966488	9.280777	Zinder, 50km NE of	Zinder	Found dead	74295112
Varanus griseus	6662*	14.833452	13.190565	Ngourti, 60km S of	Diffa	Live	74295147
	6665*	15.071482	13.219652	Ngourti, 30km S of	Diffa	Tracks	N/A
	6686*	16.564845	12.928235	Ti-N-Toumma	Diffa	Tracks	N/A
	6688*	16.555078	12.906817	Ti-N-Toumma	Diffa	Live	74295166
	6689*	16.423758	12.621273	Ti-N-Toumma	Diffa	Tracks	N/A
	6693*	16.217758	12.198542	Ti-N-Toumma	Diffa	Live	74295169
	6694*	16.12791	12.049452	Ti-N-Toumma	Diffa	Tracks	N/A
	6695	16.023922	11.860513	Ti-N-Toumma	Zinder	Tracks	N/A
	6711	15.642877	11.505478	Termit-Kaoboul	Zinder	Tracks	N/A
	6732	15.867435	11.454855	Termit, Madaranga	Zinder	Tracks	N/A

	6749	16.343802	11.463573	Termit, Nourou Nga	Zinder	Tracks	N/A
	6754	16.181423	11.356395	Termit, Louli Agadem Nga	Zinder	Tracks	N/A
	6759	15.901833	11.267952	Termit, Dioro Tiouwi Hanga	Zinder	Tracks	N/A
	6766	15.196902	10.727067	Tasker, 8km N of	Zinder	Tracks	N/A
Varanus niloticus	6622	13.67548	9.182817	Mirya, 2km SW of	Zinder	Inquiry	N/A
Cerastes cerastes	6714	15.80434	11.452587	Termit, Diora Aouoranga	Zinder	Live	74295186
	6717	15.80434	11.452587	Termit, Diora Aouoranga	Zinder	Skin shed	N/A
	6734	15.861802	11.452675	Termit, Madaranga	Zinder	Live	74295191
	6746	16.324485	11.42469	Termit, Nourou Nga	Zinder	Tracks	N/A
	6748	16.343802	11.463573	Termit, Nourou Nga	Zinder	Tracks	N/A
Cerastes vipera	6658*	14.684633	13.138393	Nguigmi, 50km N of	Diffa	Live	74295197
	6670	16.149493	13.21645	Koussa Arma well	Diffa	Tracks	N/A
Echis leucogaster	6653	13.511182	12.823018	Diffa, 30km NE of	Diffa	Skin shed	N/A

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Chapter 4

Tool development

4.1. Article II. Development of 23 microsatellite loci for Boulenger's agama (*Agama boulengeri*) with partial cross-amplification in other *Agama* species

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Abstract

Patterns of biodiversity and evolutionary processes controlling them are still poorly studied in desert biomes. Fine-scale markers could help answer some of the pressing research questions for desert biomes and Sahara in particular. Such markers are available for some large mammals and crocodiles, but not for small vertebrates. Here we present a battery of microsatellite loci developed for *Agama boulengeri*, a promising model to study evolutionary and demographic processes in the Sahara-Sahel. Loci were selected by sequencing enriched DNA libraries with 454 pyrosequencing. A total of 23 polymorphic loci were successfully amplified in four multiplex reactions. Cross-amplification of all microsatellite loci in *A. agama* and *A. boueti* was partially successful. These markers are a promising tool for assessing genetic diversity, gene-flow dynamics and demographic patterns in this group. Given the genus *Agama* is distributed throughout Africa, results presented here might also facilitate studies in other regions.

Keywords: genetic diversity, lizards, population genetics, Mauritania, mountains, Sahara-Sahel

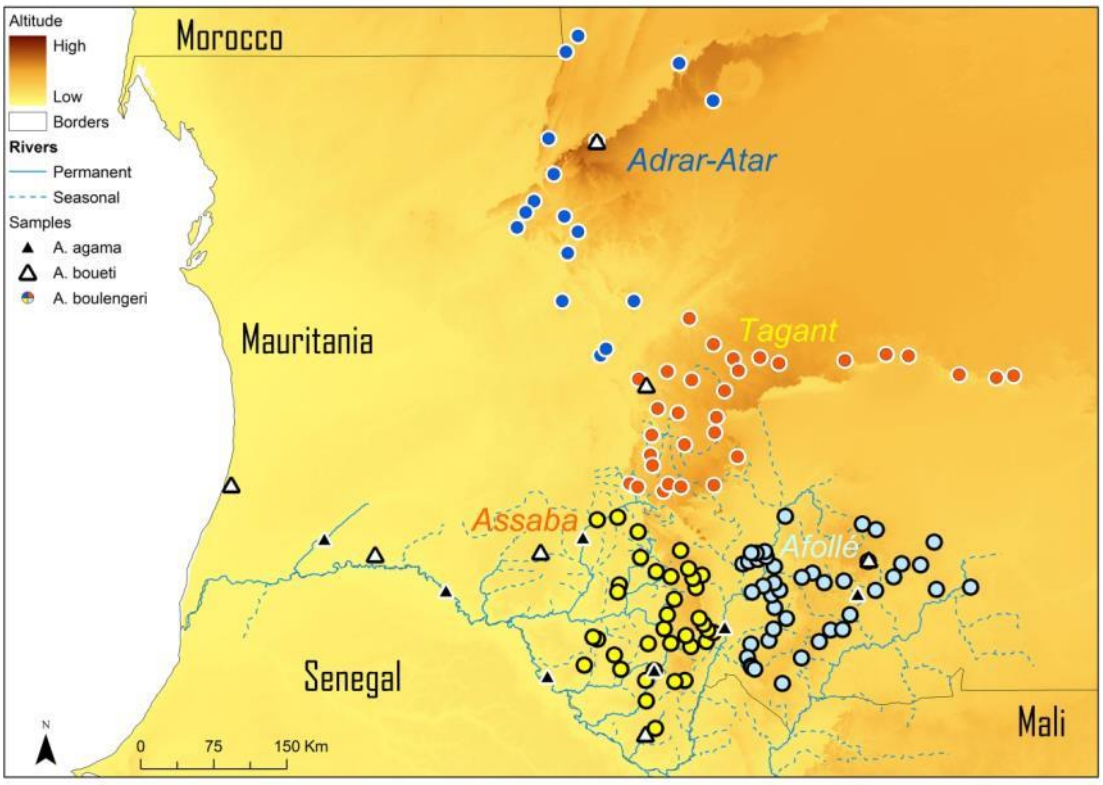

Figure 4. 1 Distribution of Agama samples used in this study. The names of the mountain systems are indicated in italics next to the respective populations. The different degrees of shading of the circles denote the groups of samples.

Desert biomes are among the least studied on the planet, particularly the Sahara where evolutionary processes and the resulting patterns of biodiversity distribution are not well understood (Brito *et al.*, 2014). Regarding herpetofauna, many biogeographic studies have been published using molecular data, but most of them focused on general phylogeographic inferences using a few nuclear and mitochondrial DNA sequences (Carranza *et al.*, 2008; Metallinou *et al.*, 2012, 2015; Wagner *et al.*, 2011). This is not surprising given the lack of basal knowledge and the scarce sampling. Nevertheless, higher resolution molecular tools like microsatellites are needed to answer many pressing research questions for desert biomes, and Sahara in particular (Brito *et al.*, 2014). Understanding the present distribution of genetic diversity, the role of the landscape and climate in shaping contact-zone and corridor dynamics, predicting the impact of climate change, and designing conservation measures at local scales all require assessing genetic diversity at a finer temporal and spatial scale. Microsatellites are prime tools for undertaking such tasks (Schlötterer, 2004; Selkoe and Toonen, 2006; Wan *et al.*, 2004). Fine scale spatial sampling is other major constraint, but fortunately the growth of molecular studies and tissue collections has led to the emergence of potential model systems, such as *Agama* lizards (Gonçalves *et al.*, 2012; Leaché *et al.*, 2014), Scincidae skinks (Carranza *et al.*, 2008), *Stenodactylus* and *Ptyodactylus* geckos (Metallinou *et al.*, 2012, 2015). Nevertheless, molecular markers for population level studies are still only available for a very reduced set of taxa (Stuckas *et al.*, 2014; Velo-Antón *et al.*, 2014).

One of the potential study models is genus *Agama*, a group of lizards very common and widespread through Africa, with a reasonably well resolved phylogeny (Leaché *et al.*, 2014). They are found in arid and semi-arid habitats that include rocky outcrops, sand deserts and forests (Le Berre, 1989; Schleich, Kastle and Kabisch, 1996). Their presence in the Mediterranean, Sahel and Sahara, usually associated with mountain ranges, makes them a prime biogeographic model to assess the influence of humid-arid cycles and the changing landscape in shaping biodiversity patterns in the region (Gonçalves *et al.*, 2012). Sequencing microsatellite markers could provide insights on ecological adaptation and possible hybridization in contact zones, the response and vulnerability of mesic reptiles and other non-flying vertebrates to climate change, the functioning of gene-flow corridors, or on genetic diversity hotspots. Conservation solutions would also be better informed by integrating such information.

The Boulenger's agama (*Agama boulengeri* Lataste 1886) is present in the Sahara-Sahel fringe, being restricted to Mauritanian mountains (Adrar Atar, Tagant, Assaba and Afollé in Figure 4.1; Padial, 2009) and a few localities in south-western Mali (Joger

and Lambert, 1996). *Agama boulengeri* is associated with arid rocky outcrops lacking vegetation cover (de La Riva and Padial, 2008; Geniez *et al.*, 2004). Given that its distribution includes a transition zone between biomes, and most of its distribution is restricted to sky-islands, *A. boulengeri* is a good candidate for studying contact-zone and gene-flow dynamics in the region.

Here we developed 23 polymorphic microsatellite loci for *Agama boulengeri*, starting from a pool of 12 specimens from different localities representative of the distribution area, and tested them for cross-amplification in other distant (Leaché *et al.*, 2014) species within the genus, *Agama boueti* and *Agama agama*. *A. boueti* occurs mostly in Western Sahel and Mauritanian mountains, and *A. agama* is part of a species group occurring mostly in NW Africa south of the Sahel. The samples covered the species' distribution in Mauritania (Figure 4.1).

Methods

DNA was extracted from tail tip tissue using QIAGEN's (https://www.qiagen.com/) EasySpin Kit, following the manufacturer's protocol for tissue samples. Microsatellite development was performed through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries (Malausa et al., 2011) at GenoScreen (http://www.genoscreen.fr/). A final set of 9889 sequences containing microsatellite motifs was obtained from which 386 primer pairs were designed. From these, we selected 50 giving preference to: 1) big repeat motifs (tri- and tetra-nucleotides) and 2) no composite repeat motifs, in order to limit scoring errors; and 3) size and annealing temperatures that allowed multiplexing of the primers. A total of 50 loci were initially selected and tested using seven samples from isolated localities covering the species' distribution. Loci were selected on the basis of reliably amplifying a product of the expected size, being polymorphic (Table 4.1), and being successfully amplified across samples. A total of 23 loci comprised the final dataset. These were used for genotyping 129 samples of A. boulengeri covering the whole distribution (Figure 4.1). In order to test the transferability of the chosen markers within the genus, we used eight samples of A. agama and eight of A. boueti (Figure 4.1).

PCRs were performed in four multiplex reactions (Table 4.1). For each locus, we used a tail primer labelled with a fluorescent dye (FAM, NED, VIC or PET), following the M13-primer genotyping protocol (Schuelke, 2000) and forward primer concentration of 1/10 of reverse and tail primers. PCR reactions were performed at a final volume of 10µL, using 5 µL of Qiagen Multiplex PCR Kit, approximately 10 ng of template DNA and 1 µM of primer mix (individual primer concentrations in Table 4.1). Conditions were

as follows: (95°C for 15)'; 9x (95°C for 30'', 57.5°C -(0.5°C/cycle) for 90'', 72°C for 30''); 31x (95°C for 30'', 53°C for 60'', 72°C for 30''); (60°C for 30'). PCR reactions were performed on Bio-Rad T100 Thermal Cyclers (www.bio-rad.com/) and genotyped on an ABI 3130xl genetic analyser (www.appliedbiosystems.com/). Allele scoring was performed using GeneMapper v4.1 (Applied Biosystems) with GeneScanTM-500 Liz as size standard.

Potential evidence for large allele dropout, null alleles and stuttering were assessed using MICRO-CHECKER v2.2.3 (Van Oosterhout et al., 2004) at each locus. Deviations from Hardy-Weinberg Equilibrium (HWE) and signs of genotypic linkage disequilibrium between loci (LD) were assessed in GENEPOP online version (http://genepop.curtin.edu.au/); for both types of test, the Holm-Bonferroni correction was applied. Observed and expected heterozygosity were computed using GenAlEx v6.501 (Peakall and Smouse, 2012). For these tests we divided the dataset into four spatial groups, one for each mountain system (Figure 4.1). This approach aimed at maximizing the number of samples in each group, and was based on the reported parapatric distribution of genetic lineages (Gonçalves et al., 2012), and the potential sub-populations predicted from the suitable area of occurrence (Vale et al., 2012). Although this delineation is debatable due to the geographical scale, the available sampling of just a couple of samples per site (no single-site population sampling) precludes grouping samples into smaller and more meaningful interbreeding populations.

Results and Discussion

All loci were successfully amplified in the extended *A. boulengeri* dataset, with missing data at the first amplification attempt below 40% in all cases (Table 4.1). The number of alleles per locus in *A. boulengeri* varied between 4 and 28 (mean of 18 alleles per locus). Observed heterozygosity varied between 0.04 and 0.94 (mean 0.51) and unbiased expected heterozygosity varied between 0.05 and 0.94 (mean 0.77). No evidence of large allelic dropout or stuttering was found across all markers. Signs of linkage disequilibrium (LD) after the Holm-Bonferroni correction were only found in AB19-AB20 and AB17-AB19 and only in Afollé. Deviations to Hardy-Weinberg Equilibrium (HWE) in all populations were found in three loci (AB5, AB6, AB26), while evidence for null alleles varied in each population (Table 4.1). This could be due to mutations in the primer anchor site, which would limit the use of some loci, especially the six with concordant evidence among populations (Table 4.1, Null=1). Alternatively, it could be attributed to Wahlund effects due to the artificial grouping of samples.

Table 4. 1. Characteristics of the 23 microsatellite loci for A. boulengeri. Locus: locus code preceded by multiplex group; Primers: forward, reverse, and respective concentration; Allele Nº: Number of alleles per locus; *Ho*: Observed heterozygosity; *He*: Expected heterozygosity; Null Alleles: fraction of populations showing evidence of null alleles.

Tail Colour	Locus	Genbank	Repeat Motif	Primers Sequence (5'-3')	(nM)	Size Range	Allele Nº	*Adrar*		*Tagant*		*Assaba*		*Afollé*		Missing data	Null Alleles
								Ho	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*		
PET	1. AB10	KX094494	ATGT	TGCTCTTTTCCTTATTCTGGG	9.5	140-230	18	0.67	0.85	0.57	0.91	0.58	0.89	0.70	0.89	32.3%	0.75
				CGTGAGTCCATTATTTGGCA	95												
FAM	1. AB31[1,2]	KX094495	CTAT	TGATCATGCATTCCCAGATG	6	240-340	22	0.93	0.92	0.69	0.92	0.67	0.91	0.65	0.88	36.9%	0.5
				AGAGGCCCAGCCATTGTAG	60												
VIC	1. AB5[1,2]	KX094496	TCTA	GCTATGGTCAGGATGACTTGC	8.5	130-210	27	0.88	0.92	0.43	0.93	0.29	0.70	0.23	0.62	17.7%	1
				TTTAAGATGGATGGCCAGATG	85												
PET	1. AB50	KX094497	TC	AACTGCCCATAGGCACTGAC	6	100-160	17	0.39	0.77	0.58	0.85	0.46	0.85	0.57	0.75	22.3%	0.5
				GCTTACATAATCCTATGTTTACACTGC	60												
VIC	1. AB6[1,2]	KX094498	TGT	CCACTGTAACTGATGCCTGC	6	90-150	21	0.63	0.90	0.58	0.90	0.28	0.92	0.55	0.93	25.4%	1
				CATGGAGCCACAAAGAGTCA	60												
NED	1. AB7[1]	KX094499	CTT	GTTCTTTAGGGCAAGTGGTCA	6	155-255	26	0.83	0.90	0.83	0.93	0.68	0.91	0.68	0.92	20%	0.25
				AACAGGGCCTTGAAGATGTG	60												
NED	1. AB8	KX094500	TGT	AGGTCTTGCTGTATTCTGGGA	8.5	100-125	4	0.11	0.15	0.29	0.30	0.04	0.40	0.00	0.09	26.9%	0.75
				TCTCATTCCAGTGTGACCCA	85												
FAM	2. AB11[1,2]	KX094501	GTT	ATTATCAAGATTCCAAGCTAAACC	12.5	230-290	6	0.27	0.30	0.19	0.66	0.22	0.42	0.17	0.42	24.6%	1
				CGAGTTACATCTGACCTTGCC	125												
VIC	2. AB15	KX094502	TCT	GTGGTTATTGCAGCAACACTG	6.5	120-220	27	0.89	0.93	0.29	0.91	0.48	0.92	0.70	0.93	26.1%	0.75
				GAACATCCTTATCCATACCTGTCA	65												
VIC	2. AB16[1]	KX094503	AGG	CAAATAGTCTAGCATGAAGGAGCA	6.5	90-130	6	0.06	0.05	0.26	0.45	0.13	0.54	0.41	0.41	36.9%	0.5
				GAGGACCATGTTGTGCCTG	65												
NED	2. AB17	KX094504	AAC	CTCAAGGAGTGATTTTCAGCA	6.5	130-200	11	0.63	0.77	0.53	0.79	0.69	0.83	0.63	0.77	6.92	0.5
				GCCAGTAACAAGACTGAAGGC	65												
PET	2. AB19[1]	KX094505	CAA	TGCTTACAAATGTAATCCATCCC	12.5	190-250	9	0.26	0.74	0.43	0.71	0.41	0.69	0.29	0.64	8.5%	0.75
				GAGCCACTCGACTTAAGAGCA	125												

PET	2. AB20	KX094506	AAC	GAATCCAGCAGACAGTATTTTCC GCCAGCTGTTAGCTCGTGTT	6.5 65	90-160	13	0.74	0.82	0.77	0.84	0.70	0.85	0.63	0.74	6.2%	0
FAM	3. AB21[1,2]	KX094507	CA	TACACCAGAAATTCTTACCCAGA ATTGAGGCAGTAGCCACTGAAA	15.5 155	230-300	16	0.43	0.78	0.38	0.89	0.48	0.78	0.46	0.76	36.8%	0.5
FAM	3. AB23	KX094508	GT	TCAAGCAACCTCTGGACACA TTTACGTCAGTAGATCATGTTCCTT	7 70	90-140	18	0.39	0.65	0.42	0.70	0.40	0.76	0.49	0.68	11.5%	0.75
VIC	3. AB24	KX094509	TCTA	CAGCGTGAAGCCAATGAAAT TCACTGAAACTGAGTGTTGGAG	7 70	180-320	27	0.61	0.92	0.74	0.91	0.75	0.93	0.82	0.94	13.8%	1
VIC	3. AB25[1]	KX094510	TCT	GGCTAGGTGGGGTTAGGAAG TGGATACCGGGGTCATACTG	7 70	130-260	28	0.94	0.92	0.50	0.90	0.68	0.93	0.63	0.94	11.5%	0.5
VIC	3. AB26[1,2]	KX094511	AC	GCATCCCCATTGCAAAAGT TTGTGCTACTCAAACTTCAGCC	7 70	90-160	24	0.57	0.85	0.32	0.77	0.46	0.92	0.50	0.88	20.8%	1
NED	3. AB27[1]	KX094512	GTT	GAGGTCTGCAAGGATGCTCA GACAAGGGTGAACCTACTGTAAAA	7 70	170-220	12	0.39	0.70	0.36	0.79	0.58	0.82	0.37	0.71	10%	0.5
FAM	4. AB32	KX094513	TTG	GCTATGCTGGATTGCAAATCACT AGTGTCAGGGTCCTGAATGG	6.5 65	190-250	11	0.40	0.84	0.39	0.83	0.42	0.89	0.34	0.85	26.2%	1
VIC	4. AB35	KX094514	CAA	TTCAGGTCACAAGGGGAACT GACTTTTGGGCACTGTCAGG	6.5 65	150-220	18	0.84	0.90	0.45	0.86	0.62	0.87	0.61	0.90	1.5%	0.5
VIC	4. AB36[1]	KX094515	AC	TTAACACAAGAAGGCATCACATC TGCTGCACTGTGTATTTCTGC	6.5 65	90-180	18	0.47	0.79	0.77	0.79	0.71	0.77	0.69	0.83	36.9%	0.25
NED	4. AB38[1]	KX094516	GT	CCTCCAGCTAGAACAGCCAC TTTTCTAGAGTGTGGGATAAGGTT	6.5 65	90-140	10	0.37	0.77	0.50	0.73	0.51	0.77	0.49	0.75	5.4%	0.75

[1]- Successfully amplified *Agama agama*;

[2]- Successfully amplified *Agama boueti*

The putative panmictic populations in the four different mountain systems cover a vast area, thus potentially masking true population dynamics, which in this case would result in an overestimation of homozygosity. Other population delimitation strategies (e.g. running clustering algorithms to further refine groups, or using 10x10Km and 25x25Km squares) and other software were tested, but did not result in clearer patterns. However, since these statistics are more representative of the issues in population delimitation than the actual quality of the markers, we still believe the reported markers are useful.

Cross amplification of the developed markers to *A. agama* and *A. boueti* was partially successful. A total of 13 and 6 loci were successfully amplified for *A. agama* and *A. boueti*, respectively (Table 4.1). Although a fraction of the 23 loci dataset in *A. boulengeri*, these species are genetically quite distinct from each other and from *A. boulengeri*. Therefore we believe the microsatellite battery presented here could be applied to other species in the genus, for instance to help species delimitation or assess invasive events by *A. agama*.

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Chapter 5

Large-scale evolutionary patterns and processes

5.1. Article III. The role of climatic cycles and trans-Saharan migration corridors in species diversification: biogeography of *Psammophis schokari* group in North Africa

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Abstract

Highlands, hydrographic systems and coastal areas have been hypothesised to form corridors across the hyperarid Sahara desert in North Africa, allowing dispersal and gene flow for non-xeric species. Here we aim to provide a genetic test for the trans-Saharan corridor model, and predict the location and stability of ecological-corridors, by combining phylogeography and palaeoclimatic modelling. The model was the *Psammophis schokari* (Schokari sand racer) group, fast-moving and widely distributed generalist colubrids occurring mostly in arid and semiarid scrublands. We combined dated phylogenies of mitochondrial and nuclear markers with palaeoclimatic modelling. For the phylogeographic analysis, we used 75 samples of *P. schokari* and *P. aegyptius*, and Bayesian and Maximum-Likelihood methods. For the ecological models, we used Maxent over the distribution of *P. schokari* and West African lineages. Models were projected to past conditions (mid Holocene, Last Glacial Maximum and Last Inter-Glacial) to infer climatic stable areas. Climatic stability was predicted to be mostly restricted to coastal areas and not spatially continuous. A putative temporary trans-Saharan corridor was identified in Eastern Sahara, with a more stable one along the Atlantic coast. Six parapatric lineages were identified within *P. schokari*, four occurring in North Africa. These likely diverged during the Pliocene. The Tamanrasset River might have been a vicariant agent. African lineages may have experienced further subsequent diversification during the late Pleistocene. The main *P. schokari refugia* were probably located along the northern margins of the Sahara, allowing its North-to-South colonisation. Trans-Saharan corridors seem to have played a role in *P. schokari* biogeography, allowing colonization of central Saharan mountains and Sahel. Some might have worked as *refugia*, and even the most stable corridors may have sections working as filters, depending on each climatic phase. We expect the use of trans-Saharan corridors to decrease for more mesic species or with less dispersal capabilities.

Keywords: climatic cycles, ecological niche-based modelling, palaeoclimate, phylogeography, snakes, vicariance.

1. Introduction

Numerous geological and climatic events have affected the geographic and biological diversity of North Africa in the last few million years (Le Houérou 1997; Fabre 2005). Geological events include the opening of the Mediterranean to the Atlantic 7-9 million

years ago (Ma), the subsequent closure 6 Ma and re-opening 5.3 Ma (MSC, Krijgsman et al., 1999), recurrent episodes of desiccation and refilling in the Red Sea area (Girdler 1991; Bosworth et al., 2005), marine transgressions (Tawadros 2011), or the Atlas mountains uplift (de Jong, 1998). These had climatic repercussions, but the most wide-ranging climatic event was a shift from tropical to arid environments around mid-Miocene (Zachos et al., 2001) that eventually led to the appearance of the Sahara desert between 7 Ma and 2.5 Ma (Schuster et al., 2006; Swezey 2009). Arid and humid conditions then alternated during the last few million years, causing a series of expansions and contractions of climatic zones (Le Houérou 1992; Swezey 2009) that largely determined current biodiversity patterns (Brito et al., 2014).

Diversification due to humid-arid cycles has been explained through genome rearrangements (Dobigny et al., 2005), adaptation to novel habitats (Boratyński et al., 2012; Carranza et al., 2002; Guillaumet et al., 2008) or, most commonly, divergence in allopatry (Brito et al., 2014). While during humid phases, the hyper-arid regions were reduced and probably isolated, in arid phases the mesic species were pushed towards coastal areas and mountains (Le Houérou 1992; Messerli and Winiger, 1992). This resulted in disjoint distributions and allopatric diversification, currently best observed in mesic species, for example Mediterranean-Sahel separations (e.g. Gonçalves et al., 2012; Guillaumet et al., 2008), isolated populations in highlands (Geniez and Arnold, 2006; Metallinou et al., 2015), rock pools (Brito et al., 2011a; Vale et al., 2015) or desert-border *refugia* (Dobigny et al., 2013). Arid phases conversely allowed range expansions of xeric taxa (Arnold et al., 2008; Kissling et al., 2016; Leaché et al., 2017; Pook et al., 2009), later broken during humid phases (Metallinou et al., 2015; Pook et al., 2009).

Landscape features modulate gene flow (Brown and Lomolino, 1998). Depending on climatic-cycle phase and species' ecological requirements, a geographic feature can constitute a barrier to gene flow, an ecological corridor (connecting two larger similar areas), a filter bridge (or barrier, a more selective connection), or a *refugium* (where a species survives during unfavourable periods). Lake Chad, for instance, has been identified as a *refugium* (Granjon and Dobigny, 2003) or a corridor connecting the Sahel to the Tibesti (Drake et al., 2011; Dumont 1982) for mesic taxa and a vicariant barrier for xeric ones (Pook et al., 2009; Metallinou et al., 2015). Mountains, presently working as biodiversity hotspots and *refugia* for mesic species (Brito et al., 2014, 2011a; Vale et al., 2015), likely constitute barriers for lowland or xeric species. Lastly, mountains, coastal areas and hydrographic systems can be linked, forming ecological corridors for mesic species. Several areas likely to constitute North-South oriented

ecological corridors have been proposed (Dumont 1982); these geographic features are hereby referred to as trans-Saharan corridors (tS-corridors), to avoid confusion with the ecological corridor feature. They include the more transitory river drainages from central Sahara Mountains (Drake et al*.,* 2011), or the more stable (thus possibly *refugia* for some species) Red Sea, Nile River or Atlantic Sahara (Brito et al*.,* 2014). However, these have been proposed based on ecological/geological data and species' distributions, and no genetic-level assessments have been conducted so far.

Our goal is to provide a genetic assessment of the validity of the tS-corridor model. For that, since most speciation events far predate the last humid/arid shift, data on the distribution of intraspecific genetic variability is required. While many mesic taxa may use the corridors during the humid phases, only those with high mobility and more adaptations to xeric environments are expected to do so during arid phases. Mesic-xeric species with broad ecological spectrum are thus expected to make more use of tS-corridors. We have selected the *Psammophis schokari* (Schokari sand racer) group in North Africa as a model since it is widely distributed in North Africa, occurring mostly in arid and semiarid habitats; it presents a continuous distribution along the Atlantic coast, thus apparently making use of this tS-corridor (Fig. 5.1). These snakes are large and mobile animals with likely good dispersal abilities. Thus, we do not expect limited dispersal or narrow distribution to restrict its use of ecologically suitable corridors. Previous work on *P. schokari* (Rato et al., 2007) identified several lineages but no conclusions were drawn regarding trans-Saharan dispersal.

Here we propose to model the species' potential distribution in different climatic phases to assess the suitability of candidate tS-corridors as ecological corridors for *Psammophis*, thus allowing us to build clear hypothesis on the persistence of gene flow along tS-corridors for mesic species during the past climatic cycles. The general aim of this study is to assess the role of corridors in trans-Saharan dispersal, with particular focus on the Atlantic Sahara tS-corridor, in an integrative framework joining phylogeography and palaeoclimatic modelling. Using *P. schokari*, we aim to answer the questions: 1) where are the areas with higher climatic stability throughout the species range and particularly West Africa and where are the potential dispersal routes across the Sahara?; 2) how is the genetic variability spatially structured?. By combining results from these two sections, we expect to find phylogeographic patterns coherent with *refugia* close to the Mediterranean coast and in the Saharan mountains, and tS-corridors connecting them.

2. Material and methods

2.1. Sampling and study areas

The snake genus *Psammophis* includes 34 diurnal fast-moving species occurring mostly throughout tropical Africa, with some species reaching the Middle East and South-Central Asia (Sindaco et al., 2013, Uetz and Hošek, 2016). *Psammophis schokari* (FORSKÅL, 1775), commonly known as Schokari sand racer, is a common colubrid occurring from West Africa to India (Fig. 5.1), mostly in desert and xeric scrublands, marginally in sandy habitats in dry Mediterranean zones (Kelly et al., 2008; Schleich *et al*., 1996; Sindaco et al., 2013). Its sister species *Psammophis aegyptius* MARX, 1958, commonly known as Egyptian sand snake, is a typical Saharan species renowned for inhabiting even the driest areas of the eastern Sahara (Baha El Din, 2006).

We used 68 samples of *P. schokari* covering a representative part of the species distribution, particularly in West Africa (Appendix A, Table A.1; Fig. 5.1). Seven samples of *P. aegyptius* were also included. For the phylogenetic analyses (see below), outgroups and other species of *Psammophis* were selected based mostly on Kelly et al. (2008). Additional *P. schokari* sequences were retrieved from GenBank. Two datasets were assembled: dataset 1, used to evaluate the phylogenetic relationships and placement within the genus of the *P. schokari* and *P. aegyptius* lineages; and dataset 2, used to date the divergence events among the lineages. Dataset 1 included all *P. schokari* and *P. aegyptius*, eleven *Psammophis* species representative of the diversity of the genus, and six outgroups (Table A.1). Dataset 2 included eleven *P. schokari* and two *P. aegyptius* specimens representative of intraspecific lineages, plus representatives of other species spanning the *Psammophis* genus and the superfamily Colubroidea, plus *Achrocordus granulatus* as outgroup, in line with previous works (Table A.1; see also section 2.5).

For ecological models, a total of 748 observations (Figs. A.6, A.7) were collected from fieldwork (n = 244), museum collections (n = 110) and bibliography (n = 394). These observations were used to create two datasets: (i) Global, with 629 records at five arc-minute resolution (~10x10 km); and (ii) Regional (Northwest Africa), with 379 records at 30 arc-second resolution (~1x1 km). A 150 km buffer around minimum convex polygons including each dataset was used to delimit two corresponding study areas (see 2.6). All spatial analyses were conducted in ESRI ArcGIS 10. In order to reduce bias from uneven sampling, and to geographically and environmentally homogenize datasets (Merow *et al*., 2013), localities were randomly removed from clusters of

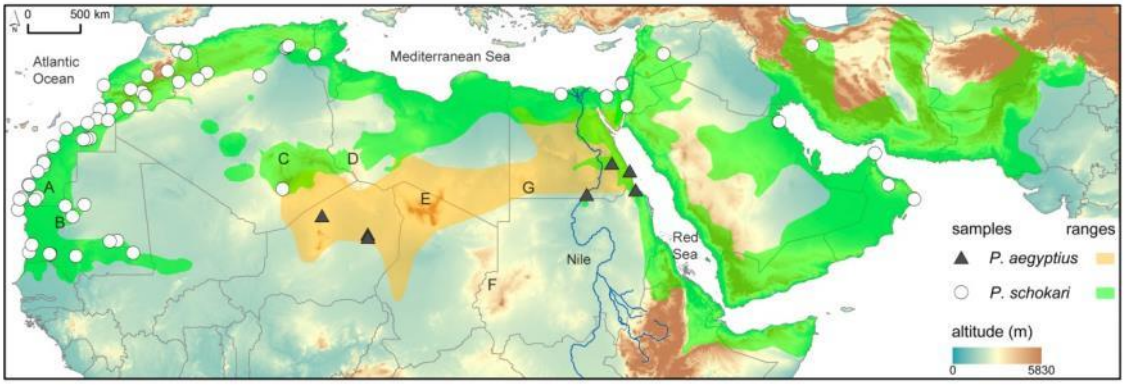

Figure 5. 1. Ranges and sample localities of P. schokari and P. aegyptius. Ranges were drawn based on distribution data from Sindaco et al. (2013), presence records collected for this study, and suitable areas predicted by ecological modelling. Letters mark mountain ranges mentioned in the text (A: Adrar Souttouf; B: Adrar Atar; C: Hoggar, Mouydir and Tassili n'Ajjer; D: Fezzan; E: Tibesti-Dohone; F: Ennedi-Borkou and Marra; G: Uweinat-Gilf Kebir).

species occurrence (e.g. Martínez-Freiría et al., 2015). The nearest-neighbour (NN) index (ArcGIS 10) was used as assessment. Low clustered distributions were obtained for both global (z-score = -19.67; NN-ratio = 0.478) and regional (z-score = -11.57; NN-ratio = 0.599) datasets, keeping 388 and 225 records, respectively (Table 5.2).

2.2. Climatic variables

Nineteen variables for current climatic conditions at 30 arc-second resolution (~1x1 km) were downloaded from WorldClim (www.worldclim.org; Hijmans et al., 2005). Variables were clipped to each study area (Global and Regional) and, for the Global dataset, upscaled to five arc-minutes (~10x10 km). After visual inspection, five variables were excluded due to the presence of spatial artefacts. The remaining 14 variables (Table A.2) were considered for ecological models. Bivariate correlations among the 14 variables were tested within Global and Regional datasets. We retained the same five slightly correlated (R<0.7) variables in both datasets (BIO 4, 10, 12, 14, 19; Table A.2), which are commonly used in ecological niche-based modelling approaches developed for other snake species (e.g. Martínez-Freiría et al., 2015; Brito et al., 2011b).

For past conditions, the same five variables were downloaded from WorldClim for the Last Interglacial (LIG; ~120,000–140,000 years BP; Otto-Bliesner et al., 2006), Last Glacial Maximum (LGM; ~21,000 years BP; Paleoclimate Modelling Intercomparison Project Phase III – PMIP3-CMIP5) and middle Holocene (midHol; 6000 years BP; PMIP3-CMIP5) periods. These periods are generally accepted as qualitatively representative of previous cycles during the Pleistocene (e.g. Martínez-Freiría et al., 2017), in spite of variations in duration and intensity, since later cycles had the largest amplitude (Snyder, 2016). LIG and midHol variables were at 30 arc-second resolution. The corresponding Global Circulation Models (GCM) were the NCAR-CCSM and

CCSM4, respectively. LGM variables were at 2.5 arc-minute resolution (~5 x 5 km) and were retrieved for three GCMs: CCSM4, MIROC-ESM and MPI-ESM-P. Past variables were also upscaled to 5 arc-minute resolution for Global models and kept at original pixel size for Regional models.

2.3. DNA extraction and amplification

DNA was extracted from ethanol-preserved tissue using DNeasy Blood & Tissue Kit (Qiagen) as per manufacturer's instructions. Amplifications were performed using MyTaq™ Mix. To benefit from sequence data available from GenBank (Kelly et al., 2008; Rato et al., 2007), specimens were bi-directionally sequenced for two mitochondrial (NADH dehydrogenase subunit 4, ND4; cytochrome *b,* CYTB) and two nuclear (oocyte maturation factor MOS, c-mos; Recombination activating gene 2, RAG2) gene fragments. ND4, CYTB, c-mos and RAG2 were amplified using primers from Arevalo et al. (1994), de Queiroz et al. (2002), Saint et al. (1998), and Vidal and Hedges (2005), respectively. PCR conditions were: pre-denaturation at 94ºC (15'); 40 cycles with 92ºC (30") denaturing, 50ºC (45") annealing and 72ºC (45") extension; final extension at 60ºC (15'). Some samples required repeating the reactions with annealing temperatures in the 48-52ºC range.

2.4. Phylogenetic analyses

Sequences were aligned using MAFFT v7 (Katoh and Standley, 2013), with Auto option, then proofread by eye. No stop codons were found in coding genes. Each marker was individually analysed inferring independent ML trees in RAxML v8.1.21 (Stamatakis 2014), in order to detect topological incongruences suggesting sample curation errors.

For both sequence datasets, the best-fit partitioning scheme and models of molecular evolution were selected using PARTITIONFINDER v.1.1.1 (Lanfear et al., 2012) with the following settings: linked branch lengths, BEAST models, BIC model selection criterion, and all partition schemes searched. Initial partitions schemes by gene and by codon were applied. Phylogenies were inferred using Bayesian and Maximum Likelihood (ML) methods. Gene and codon partitions were applied according to PARTITIONFINDER. Bayesian analyses were performed in MRBAYES version 3.2.6 (Ronquist et al., 2012) and BEAST v1.8.3 (Drummond et al., 2012). MRBAYES was run for 10^7 generations in two independent runs sampling every 1000 generations. Parameters of sequence evolution (statefreq, revmat, shape, pinvar) were unlinked for all partitions and the overall rate (ratepr) variable among them. BEAST was run in CIPRES gateway (Miller et al., 2010) in three independent runs of $5x10^7$ generations, sampling at every 5000, with

unlinked substitution and clock models, under an uncorrelated lognormal relaxed clock (Drummond et al., 2006), and considering ambiguities in nuclear sequences (manually editing the xml file to UseAmbiguities=true). A constant population size coalescent tree prior (Kingman 1982) was used for dataset 1, and a Yule speciation tree prior (Yule 1925; Gernhard 2008) for dataset 2. Burn-in was determined using Tracer v1.6 (Rambaut et al., 2014), upon stabilisation of log likelihood, average standard deviation of split frequencies, and ESS for all parameters. For BEAST analysis, runs were combined with LogCombiner and a maximum credibility tree was generated with TREEANNOTATOR (both in the BEAST package). Burn-in was determined using TRACER v1.6 (Rambaut et al., 2014). ML analyses were performed in RAXML v8.1.21 (Stamatakis 2014) through RAXMLGUI 1.5b1 (Silvestro and Michalak, 2012), with partition schemes as above and GTR+G model of sequence evolution. The program was set to perform 10 ML searches and 1000 thorough bootstrapping replicates. To test the paraphyly against the monophyly of African populations, the best tree in which Algerian/Tunisian populations are sister taxa to the rest of African and Middle East populations was compared with the alternative constrained topology in which Africa was forced monophyletic. Per-site log likelihoods were obtained with RAxML, then used to run the Shimodaira-Hasegawa (SH) (Shimodaira and Hasegawa, 1999) and Approximately-Unbiased (AU) (Shimodaira 2002) topology tests in CONSEL (Shimodaira and Hasegawa, 2001).

MEGA6 (Tamura et al., 2013) was used to calculate uncorrected *p*-distances within and among groups of samples for each mitochondrial marker in dataset 1. DNASP v.5.10.1 (Librado and Rozas, 2009) was used to calculate sequence and nucleotide diversity measures. The PHASE 2.1 algorithm (Stephens et al., 2001) implemented in DNASP was used to infer haplotypes for nuclear sequences. The software was run for 10^4 iterations with a thinning interval of five and a burn-in value of 1000, and repeated three times. Consistency was checked across runs by analysing haplotype frequency estimates and goodness-of-fit measures. Haplotypes were used to produce haplotype networks using NETWORK 5.0 (Fluxus-engineering.com) with median-joining algorithm and default parameters (Bandelt et al., 1999).

2.5. Time calibration

No calibration constraints are available within the genus *Psammophis* or the family Psammophiidae. Therefore, to obtain estimates of the divergence times among *P. schokari* lineages, the dataset was expanded to span the superfamily Colubroidea plus *Acrochordus*, and thus make use of the calibration scheme used by Wüster et al.

(2008) and Pook et al. (2009). Other authors have used a slightly different prior set (Sanders and Lee, 2008; Kelly et al., 2009), so we also dated the phylogeny according to the revised version by Sanders et al. (2010). RAG2 was excluded given that it was not available for most of the dataset.

2.6. Palaeoclimate modelling

Palaeoclimate models were computed at a Global scale, which includes the whole species distribution and aims at a species-wide average niche, and at a Regional scale, including just the West African populations and targeting local environmental species preferences. Models were generated using the maximum entropy approach with MAXENT v3.3.k (Phillips et al., 2006). This algorithm requires only presence data, performs well comparing to other methods (Elith et al., 2006), and has been used successfully in modelling snake species distributions (e.g. Brito et al., 2011b; Martínez-Freiría et al., 2015). Thirty replicates were run with random seed and 70%/30% training/testing partition and using bootstrap with replacement. Models were run with auto-features, and the area-under-the-curve (AUC) of the receiver-operating characteristics (ROC) plots was taken as measure of individual model fit (Fielding and Bell, 1997). The importance of climatic variables in explaining the species' distribution was determined by their mean percentage contribution to the models.

The individual model replicates were used to generate a mean forecast of probability of species occurrence under current conditions (Marmion et al., 2009). Standard deviation was used as indication of prediction uncertainty (e.g. Brito et al., 2011b; Martínez-Freiría et al., 2015). Individual model replicates were projected to past climatic conditions (midHol, LGM and LIG) and subjected to the same procedure. Stable climatic areas, i.e. stable potential areas of occurrence that could serve as *refugia* in different time periods (Carnaval et al., 2009), were calculated by averaging the probability of occurrence across all time phases.

Table 5. 1. Uncorrected *p*-distances among lineages, and indexes of genetic diversity for Dataset 1. Values above in regular font correspond to cytochrome *b* (CYTB), and in bold below correspond to NADH dehydrogenase subunit 4 (ND4). N = number of samples; *h* = number of haplotypes; *Hd* = haplotype diversity; π = nucleotide diversity.

CYTB **ND4**	***P. aegyptius***	***P. schokari***	Morocco	Mauritania	Algeria	Middle East	Oman	N	*h*	*Hd*	π
P. aegyptius	0.7+-0.2	9.3+-0.8	9.4+-0.9	9+-0.9	9.4+-0.9	9.5+-0.9	9.3+-0.9	6	5	0.933+-0.122	0.00747+-0.00135
	0.9+-0.2							**7**	**5**	**0.857+-0.137**	**0.00815+-0.00167**
P. schokari		4.0+-0.3						68	39	0.972+-0.009	0.04176+-0.00286
	11.3+-1.1	**4.1+-0.4**						**71**	**43**	**0.976+-0.007**	**0.04123+-0.00241**
Morocco			0.6+-0.1	4.5+-0.6	4.8+-0.6	5.8+-0.6	6.6+-0.7	32	19	0.925+-0.034	0.00484+-0.00061
	11.3+-1.2		**0.6+-0.1**					**34**	**22**	**0.957+-0.02**	**0.00580+-0.00080**
Mauritania				0.6+-0.1	5.2+-0.6	5.9+-0.6	6.5+-0.7	13	11	0.974+-0.039	0.00587+-0.00133
	11.6+-1.2		**5.1+-0.7**	**1+-0.2**				**14**	**8**	**0.857+-0.077**	**0.00949+-0.00247**
Algeria					1.2+-0.2	5.5+-0.6	6.1+-0.7	10	6	0.844+-0.103	0.01172+-0.00195
	10.1+-1.1		**5.6+-0.8**	**6.1+-0.8**	**1.1+-0.3**			**10**	**3**	**0.6+-0.131**	**0.0110+-0.00188**
Middle East						2.4+-0.2	6.6+-0.6	9	7	0.944+-0.070	0.02109+-0.00264
	11.6+-1.2		**5.4+-0.7**	**5.8+-0.8**	**5.7+-0.8**	**1.9+-0.3**		**9**	**6**	**0.889+-0.091**	**0.01939+-0.00394**
Oman							0.5+-0.2	3	3	1.000+-0.272	0.00546+-0.00231
	11.1+-1.2		**5.8+-0.9**	**7.1+-0.9**	**6.1+-0.9**	**6+-0.8**	**0.2+-0.1**	**3**	**3**	**1.000+-0.272**	**0.00192+-0.00064**

2.7. Niche overlap test

In order to assess the potential role of ecological processes in the evolutionary history of the lineages along the Atlantic corridor, we tested niche overlap, equivalency and similarity between regional lineages (see Warren et al., 2008). Tests were based on a 2D representation of climatic space of lineages (retrieved using 14 climatic variables; Table A.2), represented by the first two components of a Principal Component Analysis (PCA). We used the PCA-env ordination approach developed by Broennimann et al. (2012) in R (R Core Team 2016). This approach uses Gaussian functions to smooth densities of records and background climate and measures overlap through Schoener's *D* metric. The significance of equivalency and similarity tests is approached via randomization; we used 100 repetitions.

3. Results

3.1. Phylogenetic relationships

Most samples were successfully sequenced for all markers (Table A.1). Dataset 1 resulted in an alignment of 3111 positions (Table A.3). Uncorrected *p*-distances and diversity measures for the mitochondrial markers of *P. schokari* and *P. aegyptius* lineages can be found in Table 5.1. Genetic identity of specimens was coherent with prior morphology-based species assignment: specimens identified as *Psammophis schokari* and *P. aegyptius* formed two reciprocally monophyletic groups in the concatenated phylogenetic ML and BI trees (Fig. 5.2). The nuclear DNA did not exhibit a clear separation in the network analysis, with a shared basal haplotype in c-mos, and some RAG2 *schokari* haplotypes being closer to *aegyptius* haplotypes than to the remaining *schokari* alleles (Fig. 5.2). Six monophyletic lineages of *P. schokari* were found, three occurring in Northwest Africa with apparently parapatric distributions, one in NE Africa and the Middle East, one in Iran, and one in SE Arabian Peninsula (Oman). ML and BI supported sub-structuring was found within all four lineages occurring in Africa. Except for the earliest-splitting Oman lineage, relationships among lineages were not resolved. Monophyly of the NW African lineages was recovered in some analysis, but the topology test was inconclusive (Table A.4). A "Middle East" lineage, spreading from Egypt to the Persian Gulf, and a Mauritanian lineage occurring throughout the country were identified. The later apparently contacts with the Moroccan one. Most nuclear haplotypes of the Mauritanian lineage are shared by Moroccan populations (Fig. 5.2).

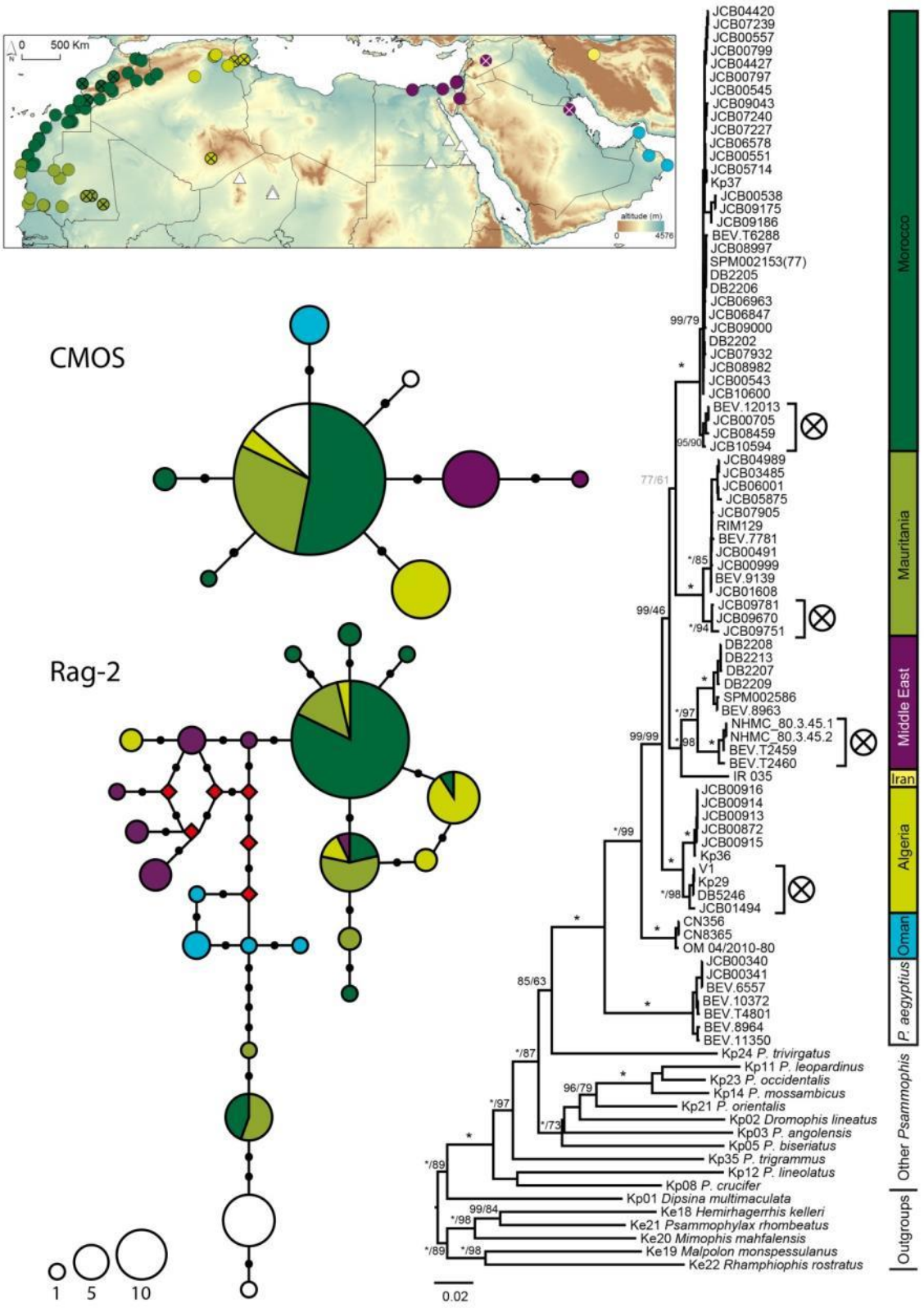

Figure 5. 2. Phylogeographic relationships among the lineages of *P. schokari* and *P. aegyptius*, and their position within the genus (RAxML and MRBAYES, dataset 1, all markers). The colours in the tree, map, and haplotype networks represent the lineages identified in the phylogenetic tree analysis. The RAxML tree is represented; posterior probability (pp) and bootstrap values (bss) are indicated as pp/bss; asterisks represent values of 100; values below 95%pp or 70bss are not shown, except for the node in light grey. Haplotype networks were calculated using the Median-Joining algorithm in NETWORK; small black dots represent mutation steps; red diamonds represent predicted missing haplotypes.

3.2. Time calibration

Dataset 2 resulted in an alignment of 2379 positions (Table A.3). Using the alternative calibration scheme by Sanders et al. (2010) produced very similar results (Appendix A, Fig. A.3). *Psammophis schokari* and *P. aegyptius* separation was dated at 9.93 Ma (7.85 – 12.07), while their estimated crown ages are 6.17 My (4.89-7.5) and 0.78 My (0.41-1.21), respectively. Divergence events among the major lineages of *P. schokari* were all placed in the Pliocene. African *P. schokari* lineages and *P. aegyptius* may have suffered roughly contemporaneous internal divergence events during the late Pleistocene.

3.3. Palaeoclimate models

Predictions of ecological models were robust (Table 5.2), identifying most of the species' occurrence with high probability (Figs. A.6-A.9). Accuracy was lower in the most arid parts of the Algerian Sahara and in portions of the Asian range. Contribution of variables changed according to modelling approach (Table 5.2).

The major areas predicted with climatic stability (potential *refugia*) in the Global model were mostly restricted to coastal areas and were not spatially continuous (Fig. 5.4A). In the Regional model, climatic stability was predicted to restricted areas of the West Sahara and in few patches across north-eastern and central Morocco and central and south-western Mauritania (Fig. 5.4B). When comparing the Moroccan and Mauritanian models, climatic stability mostly corresponded to allopatric areas (Fig. 5.4C-D). In comparison to predictions for the current time, there was a general trend for an increase of available suitable climatic areas in the LGM scenarios and the opposite trend for the mid Holocene and LIG scenarios (Figs. A.6-A.9).

Table 5. 2. Number of samples, evaluation, and variable contribution for models. Top: number of samples and area-under-the-curve (AUC) average (and standard deviation) for training and testing, and AUC standard deviation for the four distinct approaches (Global, Regional, Morocco and Mauritania). Bottom: average (and standard deviation) contribution of each variable to the respective model.

metric / variable	Global	Regional	Morocco	Mauritania
N training / test samples	265 / 113	157 / 66	139 / 59	19 / 7
Training AUC	0.891 (0.007)	0.9333 (0.005)	0.927 (0.007)	0.931 (0.022)
Test AUC	0.842 (0.017)	0.882 (0.018)	0.899 (0.011)	0.901 (0.05)
AUC Standard Deviation	0.0163	0.016	0.012	0.036
BIO 4	27.889 (2.658)	9.075 (2.36)	4.015 (1.366)	53.255 (8.985)
BIO 10	19.061 (4.26)	49.594 (6.96)	36.785 (6.935)	1.946 (2.59)
BIO 12	9.861 (2.011)	16.35 (3.965)	5.712 (3.178)	25.476 (7.31)
BIO 14	2.32 (1.138)	3.804 (1.55)	3.591 (1.22)	3.611 (3.024)
BIO 19	40.871 (2.7)	21.175 (7.04)	49.896 (6.614)	15.711 (5.59)

3.4. Niche overlap

Niche overlap between Moroccan/Mauritanian lineages is extremely reduced (D = 0.036; Fig. A.10). Their niches are not equivalent (Equivalency test, $p < 0.05$; Fig. A.10) although a similar pattern of habitat selection between lineages cannot be rejected (Similarity test, $p > 0.05$; Fig. A.10).

4. Discussion

The history of North Africa is rich in climatic and geological changes with significant impact on local biodiversity, making it hard to link timing of diversification with particular past events. Nevertheless, by combining geological/climatic knowledge and phylogenetic data, it is possible to approximate the processes that shaped Sahara colonization and the diversity of species in and around it. The models here presented include only climatic conditions, as no accurate geological data are available to past conditions for such large area. However, climatic variables should also be a reasonable surrogate for past habitat changes, as the distribution of desert species (particularly ectotherms) is strongly dependent on temperature and rainfall (Ward, 2009). Although geological features (e.g. soil type) also determine habitat and species distributions, we did not include such data in the models, as the large fluctuation in aeolian sand deposition regimes and scarce paleontological data caused by study area remoteness and stratigraphic discontinuities caused by erosion hamper predictions about past land-cover or soil types. Also, our paleoclimatic reconstructions are referent to the Pleistocene. During Pliocene, similar cycles seem to have occurred both globally (Lisiecki and Raymo, 2005) and in North Africa (Rohling et al., 2015), but uncertainties regarding the climatic conditions at the time and out-of-Pleistocene-bounds temperature variation (Snyder, 2016) limit the extrapolations of the models beyond the Pleistocene.

4.1. Migration corridors across the Sahara

Trans-Saharan corridors (tS-corridors) can be more or less permanent (i.e. active ecological corridors), with the coastal ones expected to be more permanent (Brito et al., 2014). The climatically stable areas mostly fit this prediction, with the Atlantic Sahara suggested even as potential *refugium* area. In a simple scenario, stronger gene flow along the Atlantic than from the coast to the Algerian Mountains would then be expected, but the distribution of genetic variability seems to indicate the opposite.

The same mitochondrial haplotype was found in E-Tunisia and Hoggar (Figs. 5.2, 5.4). This contrasts with the genetic differentiation commonly observed in populations from Saharan mountains, illustrated by the high rate of endemism (more than half of Sahara-Sahel vertebrate endemics; Brito et al., 2014. But see Nicolas et al., 2015). The species' broad niche can partly explain it, and this shows that the predicted level of connection of northern areas with south Algeria Mountains seems therefore sufficient to allow the species' dispersal. According to the ecological models, the link is more likely to occur under current conditions, which suggests a very recent (re-) colonization or secondary contact. The most probable path to southern Algerian mountains seems to be through Fezzan region in Libya, but a tS-corridor from there to the Sahel seems unlikely. A putative tS-corridor seems more likely in Eastern Sahara, active under LGM-like conditions (CCSM and MIROC, Fig. A.6), linking the Mediterranean to Gilf Kebir, Jebel Uweinat, Ennedi/Borkou, and possibly Tibesti and Marra mountains. However, identification of suitable areas in the Eastern Sahara is affected by the low availability of precise observational data (Fig. 5.1), resulting in less clear distribution patterns.

The Atlantic coast was the sole permanent tS-corridor for mesic species well supported and it seems to have been the most climatically stable dispersal path across the Sahara. The split of the Moroccan and Mauritanian lineages seems to contradict this pattern, but these lineages might have been separated by the Tamanrasset palaeoriver basin, which opened on the Atlantic coast in the north of present-day Mauritania (Fig. 5.4; Skonieczny et al., 2015). This river system was activated in the humid periods of the Sahara, which seem to have occurred cyclically since before the Pliocene (Rohling et al., 2015). With a drainage area comparable to the ones of Niger or Zambezi rivers (Vorosmarty et al., 2000), it could represent a barrier during humid phases, as well as constitute an unsuitable sandy expanse during warm ones. However, it is still possible the observed pattern is a coincidence, thus phylogeographic studies with other taxa are needed to verify this hypothesis. Continued isolation may have subsequently led to some niche divergence among lineages, although the observed pattern could not be dissociated from the differences in local climatic conditions (Fig. A.10). Predicted climatic connectivity may have decreased periodically (Figs. A.6, A.7), but the fact that present conditions predict the narrowest suitable area and still allow species persistence along the coast seems to indicate that, at least climatically, some gene flow is always possible. Individual Moroccan-Mauritanian models predicted that lineage contact is most likely under LGM-like conditions (Figs. A.8, A.9), similar to the Eastern Sahara corridor. The Red Sea coast was expected to be clearly represented in the palaeomodels, given that the topography allows altitudinal range shifts according to

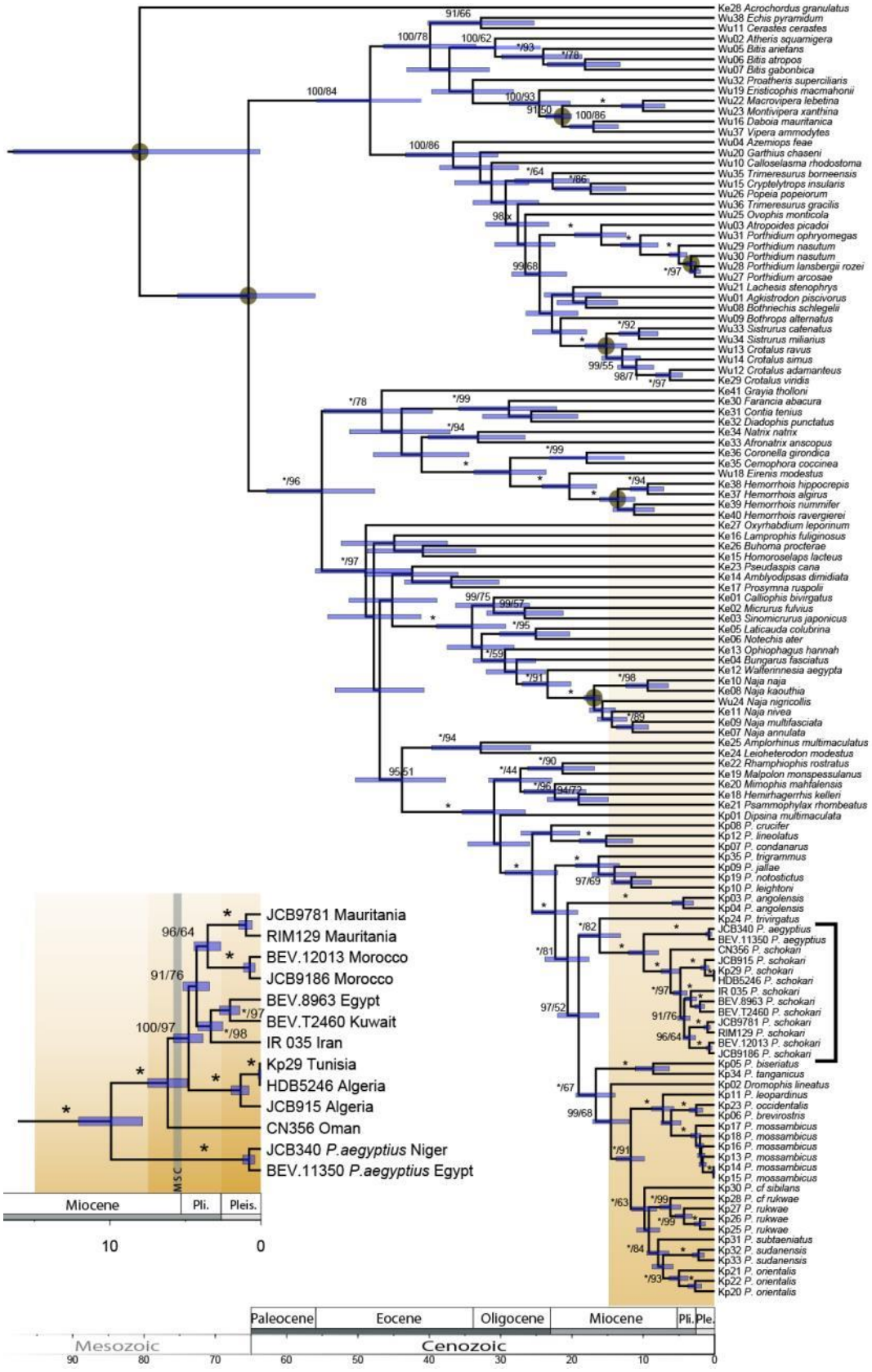

Figure 5. 3. Calibrated phylogenetic tree obtained from Dataset 2. Support values represent values from BEAST and RAxML (pp/bss); asterisks represent values of 100. Red dots mark the calibration constraints. Blue horizontal bars represent the 95% confidence intervals for age estimates. Detail of *P. schokari* and *P. aegyptius* on the left. Shadings represent (past to present): onset of aridification, oldest estimated Sahara, and youngest estimated Sahara.

climate fluctuations (Messerli and Winiger, 1992). Contrary to previously hypothesised, the Red Sea tS-corridor seems not permanent for mesic species, especially during hot and dry phases similar to LIG (Fig. A.6). Nevertheless, pixel size could be constraining model sensibility.

All considered, it seems that the Atlantic coast works as a corridor when considering the climatic cycles altogether, but certain portions (e.g. Tamanrasset river basin) may work as filters depending on the climatic phase. For example, the predicted climatic suitability of northern costal Mauritania was not mirrored by availability of observations/samples, despite considerable local sampling efforts during the last decade (Sow et al*.,* 2014), and is probably related to the unsuitable character of the wide sandy habitats present along the coast and Azeffâl dunes, in the Tamanrasset palaeoriver basin. However, Contact between Mauritanian and Moroccan populations could also be allowed through the Adrar Atar in Mauritania and Adrar Souttouf in Morocco. Assessing the detailed location of the corridors and the role of the landscape in gene-flow would however need further studies with fast-evolving markers (e.g. Velo-Antón et al., 2014) and more taxa. Still, the likelihood of a climatic corridor along the Atlantic coast is shown, and we thus expect similar genetic structure patterns to occur in other species with likely similar dispersal capabilities, like the Moila Snake (*Malpolon moilensis*) or Saw-scaled Viper (*Echis pyramidum*; Pook et al., 2008). Stronger structuring is expected for more mesic species or with less dispersal capabilities.

4.2. Phylogenetic relationships

The status of sister species is supported for *P. schokari* and *P. aegyptius*, in agreement with Rato et al. (2007), although differentiation in the nuclear markers was not complete (Fig. 5.2). Although c-mos exhibits a typical pattern of shared ancestral polymorphism where the most common haplotype in the two species is still the ancestral haplotype, RAG2 exhibits clear separation between species, so the nuclear data do not contradict reproductive isolation between these two species. The genetic distance found between the five *P. schokari* mtDNA lineages, compared in Table 5.1, was substantial (4.5-7.1%), and while not uncommon in reptiles, similarly deep evolutionary lineages were used to request taxonomy revisions in other species (e.g. *Vipera latastei*; Velo-Antón et al*.,* 2012). Still, higher resolution nuclear markers or increased genomic coverage (e.g. Velo-Antón et al., 2014) would be needed to support it and to resolve the phylogenetic relations among lineages. Additional sampling would also be necessary to define lineage distributional limits and assess gene flow in contact zones.

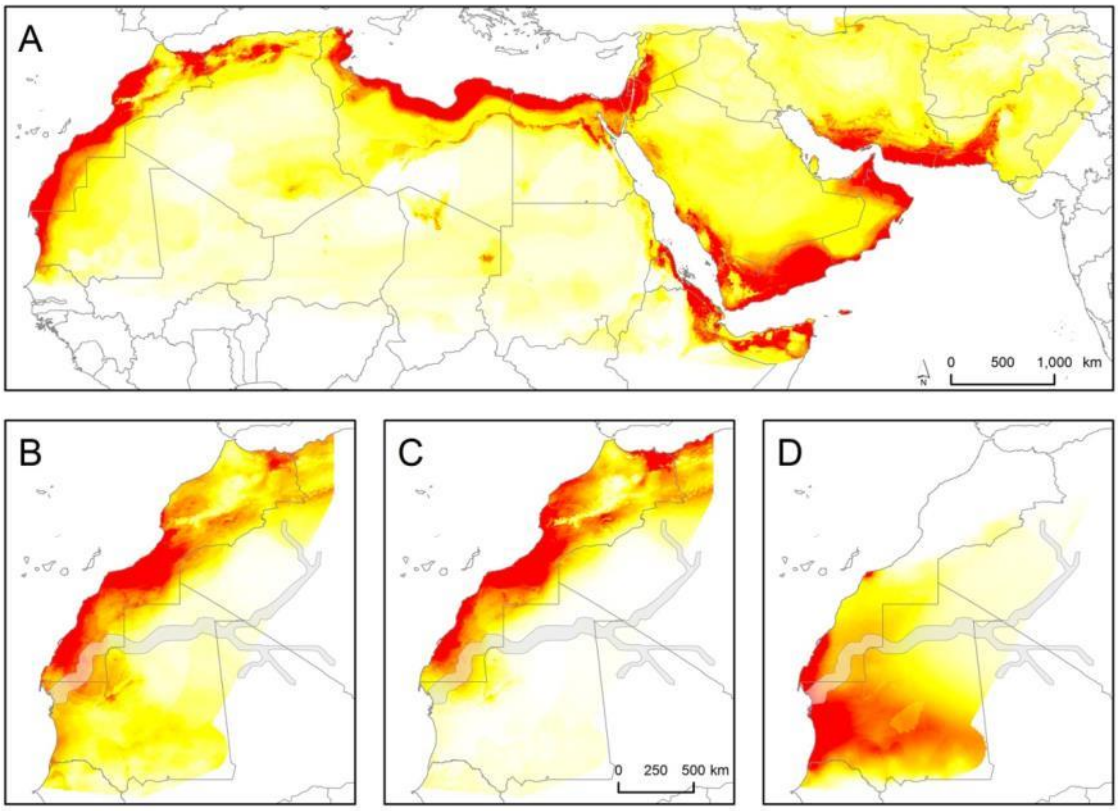

Figure 5. 4. Stable climatic areas for *P. schokari* derived from four modelling frameworks: (A) Global, all the species range; (B) Regional, the NW-African lineages; (C) Moroccan lineage only; and (D) Mauritanian lineage only. Warmer colours depict areas with higher stability. Tamanrasset paleoriver (Skonieczny *et al*., 2015) is depicted in B-D.

4.3. Biogeography and diversification

Psammophis schokari and *P. aegyptius* seem to have diverged around 10 Ma (Fig. 5.3). The latter exists only in Africa, but *P. schokari* ranges from the Atlantic coast to India, and the earliest-splitting lineage identified here is found in Oman. If the African diversity is indeed embedded within Asian diversity, an Asian origin of *P. schokari*, for instance through vicariance from African populations (the ancestor of *P. aegyptius*), is plausible. However, clarification is dependent on covering sampling gaps, since an Oman lineage migration across the Red Sea cannot be ruled out, as exemplified by other faunal exchanges between Africa and Arabia during the progressive aridification of late Miocene (Metallinou et al., 2012; Smíd et al., 2013; Tamar et al., 2016a). Ecological divergence into new more arid habitats could also have been involved, as suggested for other reptiles (Carranza et al., 2008, 2002; Metallinou et al., 2012; Pook et al., 2009; Tamar et al., 2016b).

The successive divergences among major lineages after the Messinian Salinity Crisis (Fig. 5.3) mirror the same diversification patterns in the Late Miocene and Pliocene found in many vertebrate and reptile groups (Carranza et al., 2008; Geniez and Arnold, 2006; Gonçalves et al., 2012; Wagner et al., 2011), and have been associated with the

original onset of the Sahara desert. Lineage distributions are broadly coherent with the predicted climatically stable areas, which could suggest a vicariant effect of climate (Fig. 5.4). However, our models are based on Pleistocene conditions and do not cover the Pliocene. Still, dust flux records indicate that notwithstanding a slight increase in the last Ma, humid-arid cycles have been occurring similarly since 5 Ma (Trauth et al., 2009).

The roughly contemporaneous and independent divergences of North African sub-lineages after mid-Pleistocene (Fig. 5.3) may be linked to persistence and subsequent isolation in Quaternary climate *refugia* during humid-dry cycles. In Morocco for example, the sub-lineages are separated by mountain ranges (Fig. 5.2), which agrees with a break in climatically stable areas found in Global, Regional, and Moroccan models (Figs. 5.4; A.3-A.5). This suggests a role for climate in the group diversification and matches the *refugia* patterns identified in the region (Husemann et al., 2014; Martínez-Freiría et al., 2017). Similarly, the decreased suitability predicted for north-eastern Algeria/Tunisia could be linked with the divergence of the two sub-lineages, a pattern also found in other taxa (Guiller and Madec, 2010; Husemann et al., 2014; Nicolas et al., 2015). In Mauritania, sporadic decreases in climatic connectivity or disruptive effects of rivers during humid phases (e.g. Dobigny et al., 2005) could help explain the distribution of the sub-lineages. However, given most of the phylogenetic signal is from mtDNA, effects of coalescence cannot be ruled out without employing multiple nuclear markers. The observed haplogroups in *P. aegyptius* are probably due to considerable geographic distance (isolation by distance) between sample clusters (Egypt and Niger).

4.4. Conclusions and future research

The work here presented contributes to a better understanding of how Saharan mesic corridors work according to an interplay of climatic fluctuations and geographic variability, and lays the ground for further hypothesis testing. Still, assessing how the shifts in the climatic conditions affect gene flow and evolution in other taxa is necessary to complete our understanding about the trans-Saharan corridor dynamics. Assessing the role of climate and landscape in gene-flow dynamics at local scales is also crucial. In addition to vicariance, ecological adaptation to different gradients of aridity (e.g. *Tarentola* along the Atlantic coast; Carranza et al., 2002) or habitat specialization (e.g. sympatric speciation in *Jaculus*; Boratyński et al., 2014) can also lead to species diversification. We contributed to a better understanding of the history of *P. schokari* in North Africa, but still further sampling and additional nuclear markers are needed to

understand the history of the species along its full distribution. Of particular importance are the role of the interplay of Arabia Peninsula and Africa, and the possibilities of secondary contacts between species and lineages, as well as their evolutionary and taxonomic status.

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5.2. Article IV. Assessing the role of aridity-induced vicariance and ecological divergence in species diversification in North-West Africa using *Agama* lizards

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Abstract

Diversification events in the Sahara-Sahel ecoregions of North Africa have been mostly attributed to the aridification process starting in the Miocene and subsequent arid-humid fluctuations in the Plio-Pleistocene, through vicariance or adaptation to new ecological opportunities. However, interpretations have been mostly qualitative and no study has attempted to test these contrasting hypotheses. Here we assess the importance of aridity-induced vicariance (as opposed to adaptation to novel conditions) on diversification processes in North-West African *Agama* lizards. To test the hypothesis of vicariance as the main driver of diversification, we combined dated phylogenies, species distribution models for present and past conditions, and quantitative ecological niche comparisons to verify the occurrence of three patterns expected to occur under the proposed scenario: 1) Prevalent allopatric or parapatric distributions; 2) Allopatric climatic refugia coincident with current distributions; 3) More closely related taxa showing higher niche similarity. We also assessed the distribution of genetic diversity and compared the origin and directionality of recent range expansions for two Sahelian species from different intra-generic branches, and assessed congruence with the proposed scenarios. Species and lineages occur in allopatry and/or parapatry, and the predicted climatic refugia are generally coincident with their present occurrence. Niche overlap and similarity were higher among intra-specific lineages than among species, as expected from a neutral, non-adaptive niche divergence scenario. The diffusion models for the Sahelian species identified similar points of origin for both, coinciding with the areas of highest interpolated genetic diversity and corresponding to regions of topographic heterogeneity and climatic stability. A synchronous range expansion around 320Ka was also inferred. Our results support vicariance as the main driver of diversification in NW African Agama both at large and local scales. The importance of southern Mauritania for the conservation of biodiversity and evolutionary process is highlighted.

Keywords: *Agama*, aridity, diversification, species-distribution modelling, palaeoclimate, phylogeography, Sahara-Sahel, vicariance.

1. Introduction

Global cooling and increased aridity in the late Miocene caused a significant increase in global coverage of deserts (Herbert, 2016), and created the opportunity and selective pressures for diversification and the consequent major changes in flora and fauna (Arakaki et al., 2011; Badgley et al., 2008; Cerling et al., 1997). Diversification in arid environments is commonly related to adaptation through key innovations (e.g. in cacti [Arakaki et al., 2011], or ice plants [Evans et al., 2009]) and other behavioural, morphological and physiological traits (e.g. Costa, 1995; Degen, 1997) that allowed species to occupy different climatic niches and thus diversify or even radiate in arid environments. However, diversification does not occur solely through major adaptations. Some mesic taxa with higher levels of niche conservatism (the tendency to keep the ancestral niche; Wiens et al., 2010) can also diversify in arid regions. Examples range from ecological opportunity, with slow niche divergence and the progressive occupation of newly available (drier) neighbouring conditions (Evans et al., 2009), to allopatric speciation due to the vicariant effect of aridification (Pepper et al., 2011).

In the Sahara-Sahel ecoregions of North Africa, diversification events have been attributed to the aridification of the region and the subsequent arid-humid fluctuations (Brito et al., 2014). This process started about 15Ma (mega-annums), and culminated with the appearance of the Sahara around 7Ma (Zachos et al., 2001; Zhang et al., 2014), being followed by shifts between desert- and savannah-like states at regular intervals of 20-100 thousand years (Brito et al., 2014; Le Houérou, 1997), which greatly affected species' ranges (Brito et al., 2014). Vicariance or ecological adaptation have been suggested as the main processes responsible for diversification and examples include the Sahara acting as a vicariant agent for mammals (Douady et al., 2003) and reptiles (Gonçalves et al., 2012), the adaptation to newly available arid habitats in geckos (Carranza et al., 2002), species radiations in skinks (Carranza et al., 2008), or a combination of processes (Metallinou et al., 2012). However, in most cases, biogeographical scenarios were proposed based only on coincidence of divergence times and the periods of climatic cycles (Pliocene and Pleistocene), or just empirical correlations (Brito et al., 2014). Using palaeontological data to verify past distributions and suitable climatic conditions would be an ideal solution, but unrealistic since obtaining precise data on geological and palaeo-ecological events or fossils for the region is greatly hindered by stratigraphy discontinuities caused by the erosion associated with cyclic climatic changes (Swezey, 2003). Some studies have implemented projections of past distributions to support the inference of biogeographic

scenarios concerning mesic faunal exchange across the desert (Gonçalves et al., 2018; Nyári et al., 2010), or Pleistocene climatic refugia (Martínez-Freiría et al., 2017), but they are exceptions. The integration of phylogeographic data and quantitative niche comparisons allows assessing niche conservatism and niche divergence as a proxy for the role of climate in diversification (Hua and Wiens, 2013), but, to our knowledge, no study has attempted to test the vicariant hypothesis in the Sahara-Sahel ecoregions.

Here we combine dated phylogenies, palaeomodels and ecological niche comparisons to assess the importance of aridity-induced vicariance, as opposed to adaptation to novel conditions, for species diversification in North-West Africa, using *Agama* lizards as a model. Previous studies have suggested aridity-induced vicariance as a major force behind the genus' diversification in North Africa (Gonçalves et al., 2012). With no obvious interspecific morphological variation that can be related to adaptation to different climates, their presence in the Mediterranean, Sahel and central Saharan mountains makes them a good biogeographical model to assess the influence of humid-arid cycles and the changing landscape in shaping biodiversity patterns in the region (Gonçalves et al., 2012). Agamas are common lizards, found in arid and semi-arid habitats that include rocky outcrops, deserts and forests (Le Berre, 1989; Schleich et al., 1996). Three major branches of the genus occur in North-West Africa, two of which having most of the species within the Sahara-Sahel (Leaché et al., 2014). One includes only *Agama boulengeri* LATASTE, 1886, and the other includes most species of the "Northern Africa radiation" (Leaché et al., 2014): *A. impalearis* BOETTGER, 1874; *A. boueti* CHABANAUD, 1917; and *A. tassiliensis* GENIEZ, PADIAL & CROCHET, 2011. The third branch includes the *A. agama* species group (Leaché et al., 2017), distributed along the southern fringes of the Sahel, but most of its species occur south of the study region.

If aridity-induced vicariance was the main driver of diversification of mesic taxa, three main patterns are expected: 1) Prevalent allopatric or parapatric distributions for species and lineages (Wiley, 1988) due to recurrent historical constraints to dispersal linked to humid-arid cycles. If vicariant events were non-existing and ecological divergence and adaptation were predominant, phylogenetically close groups should more readily occur in sympatry (Wiens and Graham, 2005); 2) Allopatric climatic refugia. Stable climatic areas (potential refugia) should be mostly allopatric, and coherent with present distributions (Avise, 2000). Conversely, if allopatry/parapatry was a product of adaptation to different climates (niche divergence), coincidence of climate stability with genetic structure should not be prevalent; 3) Niche similarity. If aridity-induced vicariance is prevalent, closely related clades should have similar climatic

niches, as opposed to ecological adaptation to different conditions, where closer clades are expected to have more distinct niches (Wiens and Graham, 2005). To test these hypotheses, we compared niches at the intra-specific and interspecific levels. Intra-specific comparisons were focused on *A. boulengeri* and *A. boueti*, both occurring at similar latitudes along the West Sahel and for which there was denser sampling.

Considering the humid-arid cycles mostly translated into North-South movements of the climatic regions, particularly in the southern regions of the desert, it would also be expected that under a climate driven scenario the species ranges shifted accordingly. To assess the congruence of the genetic signal with this pattern, we estimate the geographical ancestral origin of the lineages, and reconstruct range dynamics for the species present in the Sahel-Sahara fringe (*A. boueti* and *A. boulengeri*), using genetic diversity measures and continuous diffusion models that integrate genetic and spatial data.

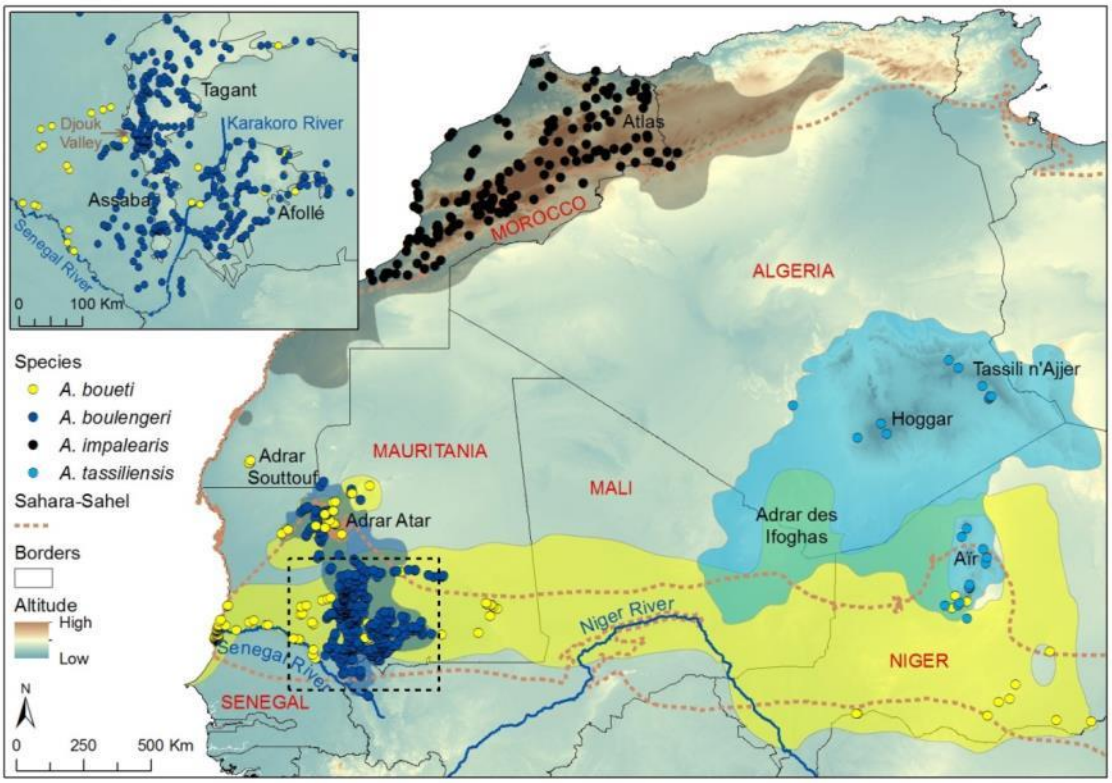

Figure 5. 5. Study area, species distribution, and presence points of *Agama* species used in this study. Species occurrence extents obtained from IUCN are represented as shaded areas. The map inset details the area of the mountains in southern Mauritania.

2. Material and methods

2.1. Phylogenetic analyses

2.1.1. Sampling and study area

A total of 718 samples of *Agama* were available for this study. Given the uneven spatial distribution of samples, an initial selection of samples best representing the geographical distribution of each species was carried out. For *A. impalearis*, since the distribution of genetic lineages had already been previously assessed (Brown et al., 2002), few additional samples were sequenced. A total of 72 were selected for *A. boueti*, 233 for *A. boulengeri*, 7 for *A. impalearis*, and 11 for *A. tassiliensis*. The limited sample size of *A. tassiliensis* was due to the difficulty of acquiring samples from the area of occurrence (see Brito et al., 2014).

2.1.2. DNA extraction and amplification

DNA was extracted from ethanol-preserved tissue using a commercially available kit (Easy-Spin). Amplifications were performed in 10 uL of 2x MyTaq™ Mix and 0.5 uM each primer. PCR conditions were: pre-denaturation at 95 ºC (15 min); 40 cycles with denaturing at 95 ºC (30 s), annealing range of 48-52 ºC (40 s), and extension at 72 ºC (45 s); and final extension at 60 ºC for 12 minutes. Some samples required minor adjustments to conditions. Four genes were amplified: ND4 (with tRNAs), 16S rRNA, NTF3 and c-mos. For the first three, we used primers from Arevalo et al. (1994), Palumbi et al. (1991), and Wiens et al. (2008), respectively. For c-mos new primers were designed based on S77/S78 of Lawson et al. (2005), and named A77 (5' – AATAGACTGGAAACAGTTGTG – 3') and A78 (5' – CCTTAGGTGTAATTCTCTCACCT – 3'). PCR products were sequenced using cycle sequencing on an automated sequencer.

2.1.3. Phylogenetic analyses

Additional sequences of *Agama* species and outgroups were selected based mostly on Leaché et al. (2014) and retrieved from GenBank (Appendix B, Table B1). DNA sequence alignments were inferred with MAFFT v7 (Katoh and Standley, 2013), with default parameters and the Q-INS-i option, then proofread and edited by eye. Coding genes (ND4, NTF3 and c-mos) were translated and no stop codons were found. Independent Maximum Likelihood (ML) trees were inferred for each marker using RAxML v8.1.21 (Stamatakis, 2014), and no topological incongruences were found. Concatenated duplicate haplotypes were removed from the alignment. The most

appropriate models of molecular evolution and best-fit partitioning scheme were selected using PARTITIONFINDER v.1.1.1 (Lanfear et al., 2012). Settings were: linked branch lengths, BEAST models, BIC model selection criterion, and all partition schemes searched. An initial partition scheme by gene (ND4 and tRNAs separated) was used.

Phylogenies were inferred with Bayesian Inference (BI) and ML methods using MRBAYES v3.2.6 (Ronquist et al., 2012) and RAXML v8.1.21 through RAXMLGUI 1.5b1 (Silvestro and Michalak, 2012), respectively. Gene partitions were applied according to PARTITIONFINDER results. MRBAYES ran for $2x10^7$ generations in two independent runs sampling every 1000 generations. Parameters of sequence evolution (statefreq, revmat, shape, pinvar) were unlinked for all partitions and the overall rate (ratepr) variable among them. Burn-in was determined using TRACER v1.6 (Rambaut et al., 2014), upon stabilisation of log likelihood, average standard deviation of split frequencies, and ESS for all the parameters. RAXML used the same partition scheme and the GTR+G model, with 10 random addition replicates and 1000 thorough bootstrapping replicates. To avoid ad-hoc intra-specific lineage delimitation, we used bPTP with both ML and BI trees, using mitochondrial markers and excluding non-Agama sequences.

Nuclear haplotypes were inferred using PHASE 2.1 (Stephens et al., 2001), implemented in DNASP. PHASE ran for 10^4 iterations with a burn-in value of 1000 and a thinning interval of five. Haplotype networks were produced using TCS V1.21 (CLEMENT ET AL., 2000) with gaps treated as missing data and otherwise default parameters. Graphic representations were obtained using tcsBU (Santos et al., 2015). Uncorrected *p*-distances within and among species and lineages were calculated in MEGA6 (Tamura et al., 2013) for each mitochondrial marker.

2.1.4. Time calibration

In order to perform the time calibration, representatives of each species and supported lineage were selected (considering sequence length) based on the results of the ML+BI phylogenetic analyses (Appendix B, Fig. B1), resulting in a total of 118 sequences, including outgroups (Table B1). We used the same calibration points as Leaché et al. (2014), which were derived from previous studies using fossil calibrations of squamates (Wiens et al., 2006) and pairwise sequence divergence calculations in an agamid species (Macey et al., 2006). Analyses were run using BEAST v1.8.3 (Drummond et al., 2012) in CIPRES gateway (Miller et al., 2010). We performed three independent runs of $5x10^7$ generations sampling every 5000, using unlinked substitution and clock models, and an uncorrelated lognormal relaxed clock (Drummond et al., 2006). We

used a Yule speciation tree prior (Gernhard, 2008; Yule, 1925), and treating ambiguities in nuclear sequences as informative sites (setting the option useAmbiguities as "true" in the XML file) . Burn-in was determined using TRACER v1.6 (Rambaut et al., 2014). Runs were combined with LOGCOMBINER and a maximum credibility tree was generated with TREEANNOTATOR (both in the BEAST package).

2.1.5. Genetic diversity and spatial diffusion models

Sequence and nucleotide diversity measures, and demographic statistics were calculated in DNASP v.5.10.1 (Librado and Rozas, 2009) for all markers. Spatially explicit representations of genetic diversity were produced using a predefined radius-search around each sample in order to create a pseudo-population from which it estimates diversity. The method was described in Veríssimo et al. (2016). The resulting diversity scores can then be spatially interpolated, in this case using the Kriging function in ArcMap. We have used a radius of ~100km, in order to include the isolated samples that would otherwise be ignored for having no neighbours.

Bayesian spatial diffusion models using a continuous phylogeny were produced using BEAST. These models use geographical coordinates of samples as continuous traits to reconstruct the geographical origin and expansion of organisms across a continuous landscape along time, and have already been implemented for predicting the origin of lineages' ancestors (Gutiérrez-Rodríguez et al., 2017; Leaché et al., 2017; Veríssimo et al., 2016). Two independent models were generated for *A. boueti* and *A. boulengeri*, using all the unique concatenated haplotypes from each species. A Cauchy Relaxed Random Walk (RRW) model (Lemey et al., 2010), and a coalescent constant population size were used as priors. MCMC chains were run for $5x10^7$ generations sampling every 5000. Runs were evaluated and processed as above. Output trees were fed into SPREAD (Bielejec et al., 2011) in order to create a spatial representation of the lineages' spread through time.

2.2. Ecological niche analyses

2.2.1. Presence data

To develop ecological-niche based models, a total of 1063 observations (169 *A. boueti*, 542 *A. boulengeri*, 228 *A. impalearis* and 124 *A. tassiliensis*) with ≤ 1km resolution (WGS 1984 datum) were collected from fieldwork (n = 889), and museum databases (n = 174). A 50 km buffer around a minimum convex polygon including all samples was used to delimit the study area. All spatial analyses were conducted in ESRI ArcGIS 10. In order to reduce spatial bias in the ecological models due to uneven sampling (Merow

et al., 2013), localities were removed at random from clusters of species occurrence, forcing a point-free minimum radius of 5km around each kept presence. The final dataset included 96 presence points for *A. boueti*, 259 for *A. boulengeri*, 152 for *A. impalearis*, and 66 for *A. tassiliensis*.

2.2.2. Climatic variables

Nineteen variables representing present climatic conditions were downloaded from WorldClim (www.worldclim.org; Hijmans et al., 2005) at 30 arc-second resolution (~1x1 km at equator). Variables were then clipped to the study area and upscaled to 2.5 arc-minutes (~5x5 km at equator). Given these layers were derived through interpolations, and represent macro-climatic conditions, this pixel-size allows decreasing computation time without affecting inference power. Five variables were excluded due to the presence of obvious spatial artefacts. To identify the significantly correlated variables, pairwise correlation among the remaining 14 was calculated using the Band Collection Statistics in ArcGIS and a threshold of R=0.7. BIO1+BIO6 and BIO2+BIO5 were all kept in spite of slightly higher correlation (R=0.83 for both pairs), due to the potential relevance of those variables for restricting the distribution of ectotherm. The final set contained BIO 1, 2, 5, 6, 7, 12 and 14 (Table 5.4).

The correspondent variables were downloaded from WorldClim for the past periods of middle Holocene (midHol; 6000 years BP; PMIP3-CMIP5), Last Glacial Maximum (LGM; ~21,000 years BP; Palaeoclimate Modelling Intercomparison Project Phase III – PMIP3-CMIP5), and Last Interglacial (LIG; ~120,000–140,000 years BP; Otto-Bliesner et al., 2006). LGM variables were at 2.5 arc-minute resolution (~5 x 5 km) and were retrieved for all three Global Circulation Models (GCM) available: CCSM4, MIROC-ESM and MPI-ESM-P. LIG and midHol variables were at 30 arc-second resolution. For LIG the GCM was NCAR-CCSM; for midHol we obtained the same GCM as for LGM. LIG and midHol layers were also upscaled to 2.5 arc-minute resolution.

2.2.3. Paleoclimate modelling

Ecological modelling was performed in BIOMOD2 (Thuiller et al., 2016), using two machine learning (artificial neural networks, ANN and maximum entropy, MAXENT) and two regression-based techniques (generalized additive models, GAM and generalized linear models, GLM). This approach aims at reducing uncertainties that may affect a given modelling technique (Wiens et al., 2009).

The study area was as described in 2.1, which also encompasses the expected variation in Sahara-Sahel extension. Since the remoteness of the study area precludes

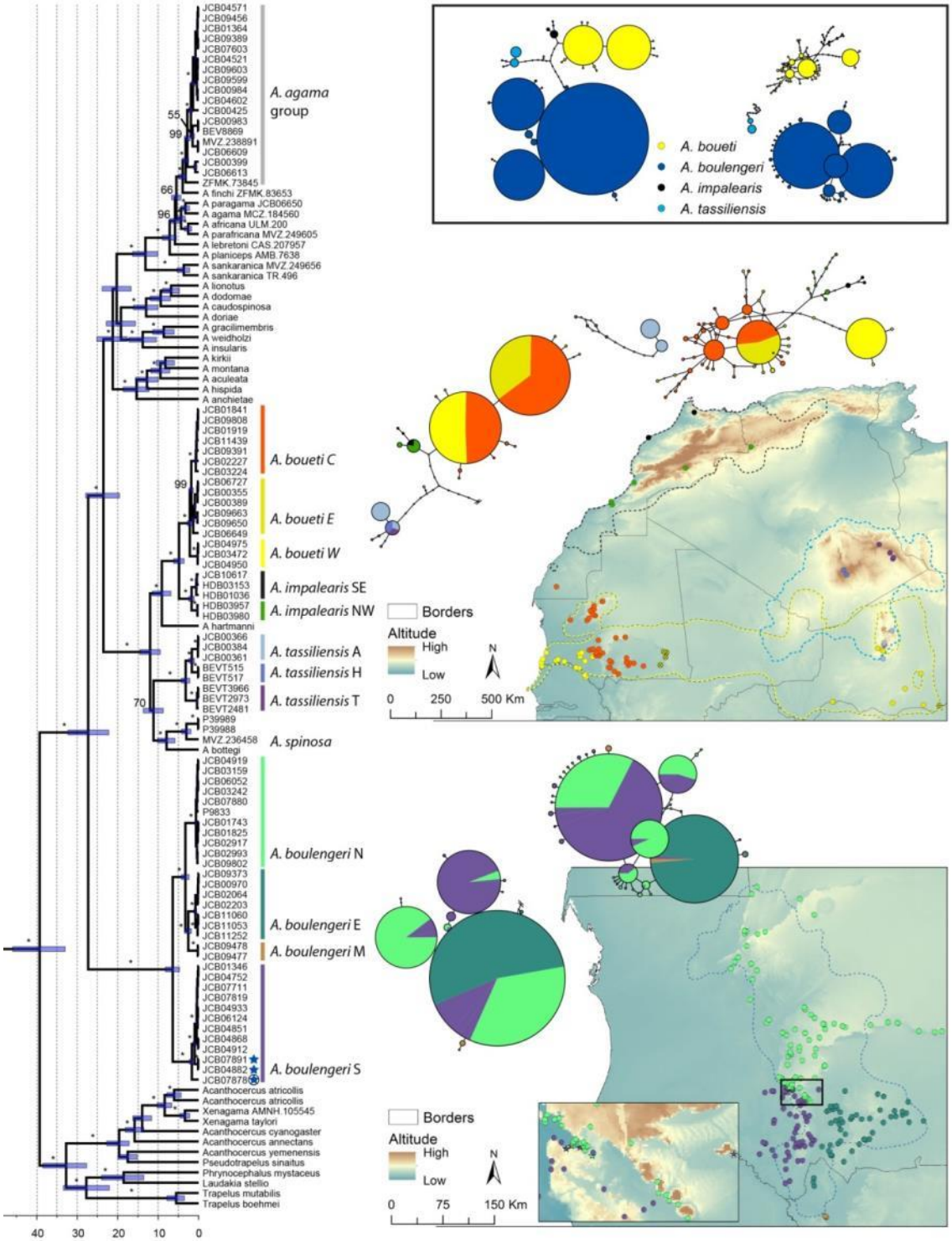

Figure 5. 6. Phylogeographic relationships among species and lineages. LEFT: dated phylogenetic tree of combined mtDNA (ND4 and 16S) and nuclear (c-mos and NT3) data, calculated with BEAST; Bayesian posterior probability (bpp) support values (%) are indicated next to the nodes, asterisks representing 100% support; some support values for terminal nodes were not represented to improve clarity; species and lineages (capital letters) are indicated next to the samples. CENTRE: Nuclear haplotype networks of c-mos (left) and NTF3 (right) obtained from TCSBU, representing the relations among the lineages (next to maps) and species (bottom of figure); colours in the bottom network correspond to those in Fig. 5.5, and in all the other networks to the lineages in the tree; each circle represents a different haplotype, and size is proportional to the number of samples sharing that haplotype; the smallest circles along the lines represent mutated positions. RIGHT: Nuclear haplotype networks coloured according to species (top); and maps representing the distributions of the lineages; dashed lines represent the extent of occurrence of the species and are colour-coded according to Fig. 5.5.

extensive sampling, we used random pseudo-absences. To control for potential bias introduced in this step, 10 independent sets of 10^4 pseudo-absences were generated using the "disk" (buffer) function with 50km around presence points. Given the large average distances between the presence points used for modelling, the relatively large buffer-size should ensure that pseudo-absences correspond to actual absences.

Ten replicates were run for each technique and pseudo-absence set, in a total of 400 replicates. Presence data for training and testing models were randomly selected for each replicate using a respective ratio of 70-30%. Replicate performance was evaluated using the TSS metric (true skill statistic; Allouche et al., 2006) and a threshold of 0.75. The threshold was selected after visualising the output, as a compromise between performance, geographical coherence with species distribution, and representativeness of modelling techniques. The consensus model was generated by averaging individual replicates (Marmion et al., 2009), and agreement among replicates was assessed using the standard deviation (Thuiller et al., 2009). Individual model replicates were projected to past climatic conditions (mid-Hol, LGM and LIG). Projections were assessed using clamping masks, which inform of environmental conditions outside of the current range of climatic conditions (Elith et al., 2010). Consensus models and standard deviation were calculated as described above. The consensus models for present and past conditions were then averaged in order to identify climatically stable areas, that is, potentially persistent areas of occurrence that could serve as refugia through time (Carnaval et al., 2009).

2.2.4. Ecological niche comparisons

Ecological niches were compared at three different phylogenetic levels, in order to evaluate if niche divergence followed a Brownian (neutral) motion: intra-generic branch (*A. boulengeri* vs. the boueti-impalearis-tassiliensis group), species, and intra-specific lineage. The later were focused on *A. boulengeri* and *A. boueti*. We used the same climatic layers used in the niche models and the PCA-env approach developed by Broennimann *et al.* (2012) and updated with functions from ECOSPAT R package (Broennimann et al., 2016). This method uses a Principal Component Analysis (PCA) to create a two-dimensional representation of climatic space, on which it performs comparisons between pairs of entities, in this case defined by minimum-convex-polygons encompassing lineage and species' distributions. Overlap was measured using the *D* metric (Warren et al., 2008), following Broenniman et al. (2012). Both equivalency and similarity tests (Warren et al., 2008) were run with 500 replicates. However, it should be noted that equivalency tests are more restrictive and affected by

allopatric ranges, thus typically less adequate when addressing biogeographical questions (Peterson, 2011).

3. Results

3.1. Phylogeography

A total of 374, 325, 377 and 298 samples were successfully sequenced for 16S (522bp, aligned), ND4 (699bp coding portion plus 195bp of tRNAs), c-mos (570bp) and NTF3 (669bp), respectively (Table B1). From those, 341 unique concatenated sequences were kept for the concatenated tree (Fig. B1). PartitionFinder model selection is summarised in Table B2.

Table 5. 3. Summary genetic diversity and demographic statistics for mitochondrial markers, based on the 341 samples used to construct the phylogenetic tree shown in Fig. B1. L = minimum length, excluding sites with gaps and missing data; P = number of polymorphic sites, excluding sites with gaps and missing data; N = number of samples or phased sequences; *h* = number of unique haplotypes; *Hd* = Haplotype diversity; π = nucleotide diversity; R2 = Ramos-Onsins and Rozas R2 statistic; *D* = Tajima's *D* (significant values in bold font); F_S = Fu's F_S statistic.

	16S								
Group	L	P	N	*h*	*Hd*	π	R2	D	F_S
A. boueti	408	26	64	24	0.941+-0.014	0.01001+-0.00053	0.1028	-0.24999	-9.61
boueti C	473	11	37	14	0.926+-0.018	0.00407+-0.00030	0.0896	-0.82855	-7.528
boueti E	411	19	13	7	0.833+-0.086	0.00624+-0.00112	0.1498	-0.02084	-1.55
boueti W	473	10	14	4	0.495+-0.151	0.00116+-0.00041	0.1246	-1.27826	-1.727
A. boulengeri	392	8	165	30	0.895+-0.011	0.04042+-0.00077	0.1408	1.6867	3.75
boulengeri E	393	3	46	5	0.558+-0.054	0.00296+-0.00028	0.1151	0.05554	0.226
boulengeri N	434	56	62	14	0.699+-0.060	0.00331+-0.00042	0.0573	-1.25047	-7.648
boulengeri S	474	5	54	10	0.725+-0.042	0.00399+-0.00080	0.0656	-1.15259	-2.036
A. impalearis	467	0	8	3	0.464+-0.200	0.00749+-0.00301	0.1735	0.04082	2.952
A. tassiliensis	456	12	13	5	0.769+-0.099	0.01749+-0.00324	0.196	1.01511	4.223
	ND4+tRNAs								
Group	L	P	N	*h*	*Hd*	π	R2	D	F_S
A. boueti	801	107	64	41	0.977+-0.008	0.02605+-0.0018	0.0926	-0.32854	-6.421
boueti C	802	41	37	21	0.946+-0.022	0.0068+-0.00077	0.0615	-1.586	-7.789
boueti E	801	48	13	11	0.974+-0.039	0.01793+-0.00301	0.1345	-0.32067	-1.031
boueti W	811	10	14	9	0.912+-0.059	0.00301+-0.00043	0.0987	-0.87291	-3.968
A. boulengeri	763	199	165	8	0.979+-0.005	0.07477+-0.00157	0.1425	1.47943	-2.534
boulengeri E	802	43	46	24	0.874+-0.045	0.01175+-0.00077	0.1003	-0.34733	-4.043
boulengeri N	797	42	62	32	0.949+-0.015	0.00795+-0.00046	0.0703	-0.96856	-14.239
boulengeri S	765	75	54	29	0.956+-0.015	0.01062+-0.00194	0.05	-1.78865	-8.721
A. impalearis	785	40	8	6	0.929+-0.084	0.02106+-0.00470	0.1818	0.3845	1.978
A. tassiliensis	799	66	13	7	0.795+-0.109	0.03007+-0.00711	0.1787	0.50935	5.767

Monophyly of all species was confirmed, and no nuclear haplotype sharing was detected among them (Fig. 5.6), supported the phylogenetic relationships described in previous studies. Estimated species crown ages spanned the Pleistocene (*A. boueti*, *A. impalearis*), the Pliocene (*A. tassiliensis*), and the Miocene (*A. boulengeri*). The bPTP lineage delimitation (Fig. B2) was consistent between the ML and BI trees, recovering two lineages within *A. impalearis* ("NW" and "SE", for intercardinal directions), five within *A. boueti* ("C" [central], "W", and 3 monophyletic ones grouped under "boueti E"), three within *A. tassiliensis* ("A", "H", and "T", the initials of Aïr, Hoggar, and Tassili mountains), and six within *A. boulengeri* ("N", "S", "E", "M" in Mali; the two other lineages include three samples in the northern distribution of "S" and are sister lineages to it with a relatively recent split, thus were included under the same monophyletic "S").

The intra-specific lineages share nuclear haplotypes at varying degrees, with the exception of boueti W and tassiliensis A and T for NTF3 (Fig. 5.6). All intra-specific lineages are seemingly parapatric or allopatric (Fig. 5.6), in agreement with the first expected scenario. The lineages of *A. boulengeri* occur in separate mountain systems in Mauritania and nearby Mali. A similar pattern is suggested for *A. tassiliensis*, with one lineage in each of three central Saharan mountain systems (Tassili N'Ajjer and Hoggar in Algeria, and Aïr in Niger). *Agama boueti* lineages have a predominantly E-W distribution along the Sahel, although the boueti C lineage reaches from the Senegal River in the south up to Adrar Souttouf in Morocco in the north. All major Sahelian species' lineages occur in the Assaba-Tagant-Afollé region in Mauritania.

Diffusion models identified the same area around Tagant, Assaba and Afollé in Mauritania as the origin of dispersal of the extant diversity of both *A. boueti* and *A. boulengeri*. A second, younger possible ancestral area was depicted in Niger for *A. boueti*. Both species began marked range expansions out of the ancestral area in Mauritania at ca. 320Ka, particularly towards the northern parts of their current

Table 5. 4. Climatic variables used for ecological models and projections, global and regional variation.

code	name	units	global	regional
BIO 1	Annual Mean Temperature	ºC	-15.5 - 31.9	3.5 - 30.8
BIO 2	Mean Diurnal Range	ºC	5.8 - 20.7	4.5 - 18.5
BIO 5	Max Temperature of Warmest Month	ºC	2.9 - 48.9	22.6 - 48.9
BIO 6	Min Temperature of Coldest Month	ºC	-33.5 - 23	-12.4 - 18.8
BIO 7	Temperature Annual Range	ºC	11 - 47.8	10.9 - 42.8
BIO 12	Annual Precipitation	mm	0 - 2769	2 - 1401
BIO 14	Precipitation of Driest Month	mm	0 - 42	0 - 24

distribution (Fig. 5.7). *Agama boueti* also showed another more recent expansion towards West, the current range of the lineage boueti W. The genetic diversity of boueti C and boulengeri N lineages shows signs of recent demographic expansion, although values were not significant (Tables 5.3, B3). The spatial interpolation of diversity showed a concordant pattern of steady decrease from the centre towards the borders of both species' distributions (Fig. 5.7).

3.2. Paleomodelling

The climatically stable areas (Fig. 5.8) were generally coincident with the current species' distributions (Fig. 5.6), except for *A. boulengeri*, with the southern and eastern parts of most of its current area of occurrence, the mountains of Mauritania, appearing as less suitable than the neighbouring areas. For intra-specific variability, climate stability is roughly concordant with the present distribution of lineages in the impalearis-boueti-tassiliensis group. Two major highly-stable areas can be observed with the same NW-SE disposition as the *A. impalearis* lineages. For *A. boueti*, the identified stable areas in SW Mauritania, Senegal River mouth, and SE Mauritania are roughly concordant with the C, W, and E (in Mauritania) lineages, respectively. In the case of *A. tassiliensis* the southern stable area corresponds to the distribution of lineage A, while the northern stable area is coincident with lineages T and H, with the cores of Tassili N'Ajjer (T) and Hoggar (H) also having higher stability than the surrounding area. The LIG was predicted to be the least favourable period for all species. LGM also seems to have been overall less suitable for *A. boulengeri* (Fig. B3). Model evaluation scores and environmental variable contributions are summarised in Table B4.

3.3. Ecological niche comparisons

Niche overlap (Table 5.5) was mostly above 10% for intra-specific lineages (average 11% in *A. boueti* and 30% in *A. boulengeri*), and decreased as phylogenetic distance increased (average 3.1% among species, 1% between intra-generic branches). All values were generally low, especially considering they were weighted by the density of occurrence of each entity within the climatic space, which is likely due to the allopatry among compared entities. Similarity tests were significant among lineages of *A. boulengeri* and, although not significant, showed the same tendency for *A. boueti* lineages, except the E vs. W comparison. The remaining tests were non-significant. Using an extended climatic layer dataset, only comparisons against boueti W were non-significant (Table B5). Tests for significant dissimilarity were all negative (Table B5).

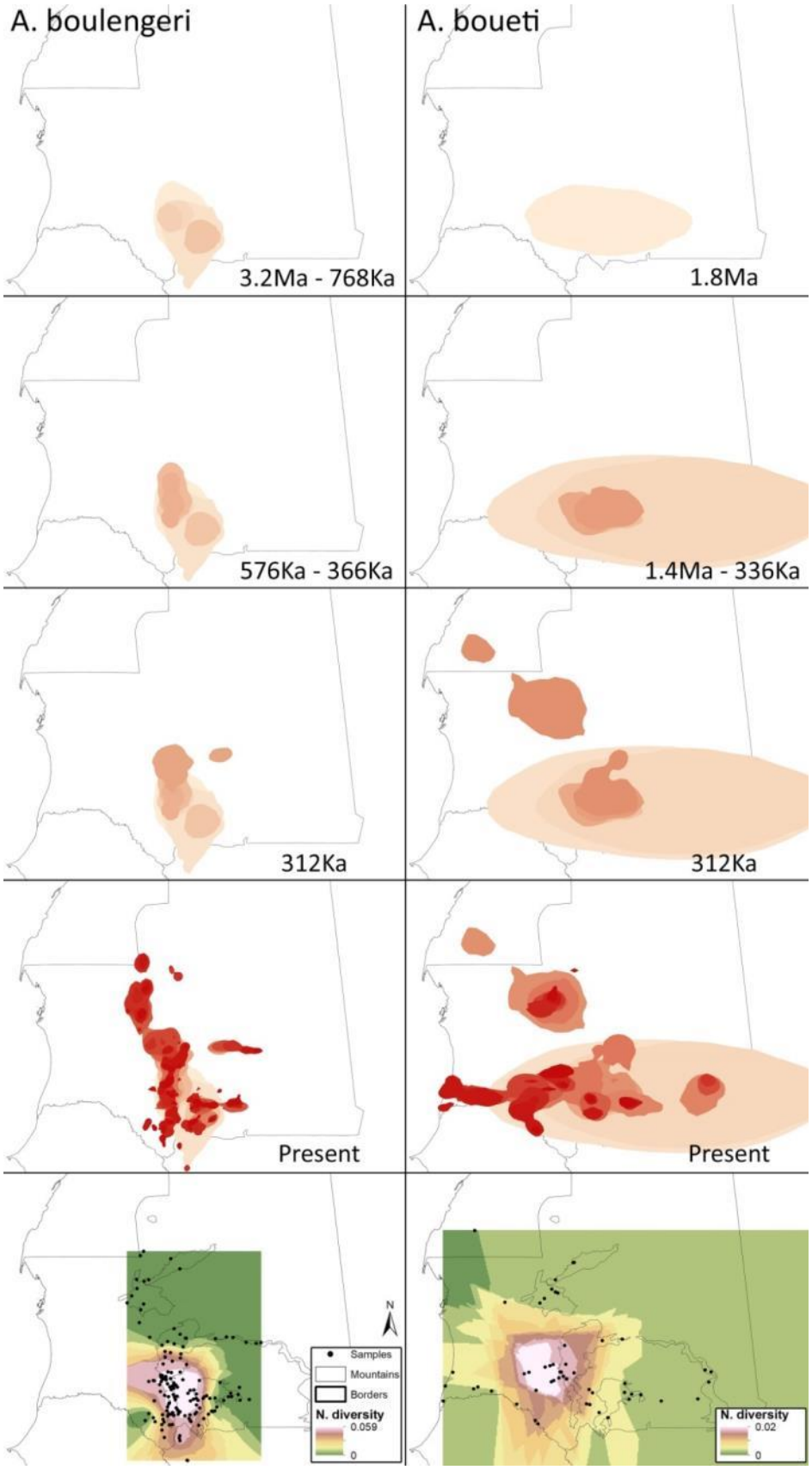

Figure 5. 7. Top four lines: continuous phylogeny spatial diffusion models for *A. boulengeri* and *A. boueti*; only the polygons representing the 80% occurrence confidence interval are shown; polygon colour represents relative age, the darker being more recent; the estimated origins for each polygon can be found in the raw output, which can be visualized in GOOGLE EARTH (pending Dryad code). Bottom: distribution of genetic diversity; coloured areas represent the nucleotide diversity calculated for A. boueti (left) and A. boulengeri (right) based on the mitochondrial markers.

4. Discussion

All the patterns expected in an aridity-induced vicariance scenario were present, supporting the role of climate in the diversification of the North-African radiation group and *A. boulengeri*, which most likely occurred due to increased and cyclic aridity for the Saharan-Sahelian populations. The mountains in southern Mauritania are shown as a diversity hotspot and predicted climatic refugium for the local *Agama* species, thus possibly for other species with similar climatic requirements, which stresses the importance of the region in terms of biodiversity conservation. The apparent ability of *Agama* from the Sahel-Sahara fringe to survive in that area during major climatic fluctuations underlines its pivotal role in the future survival of mesic species in face of the ongoing global warming.

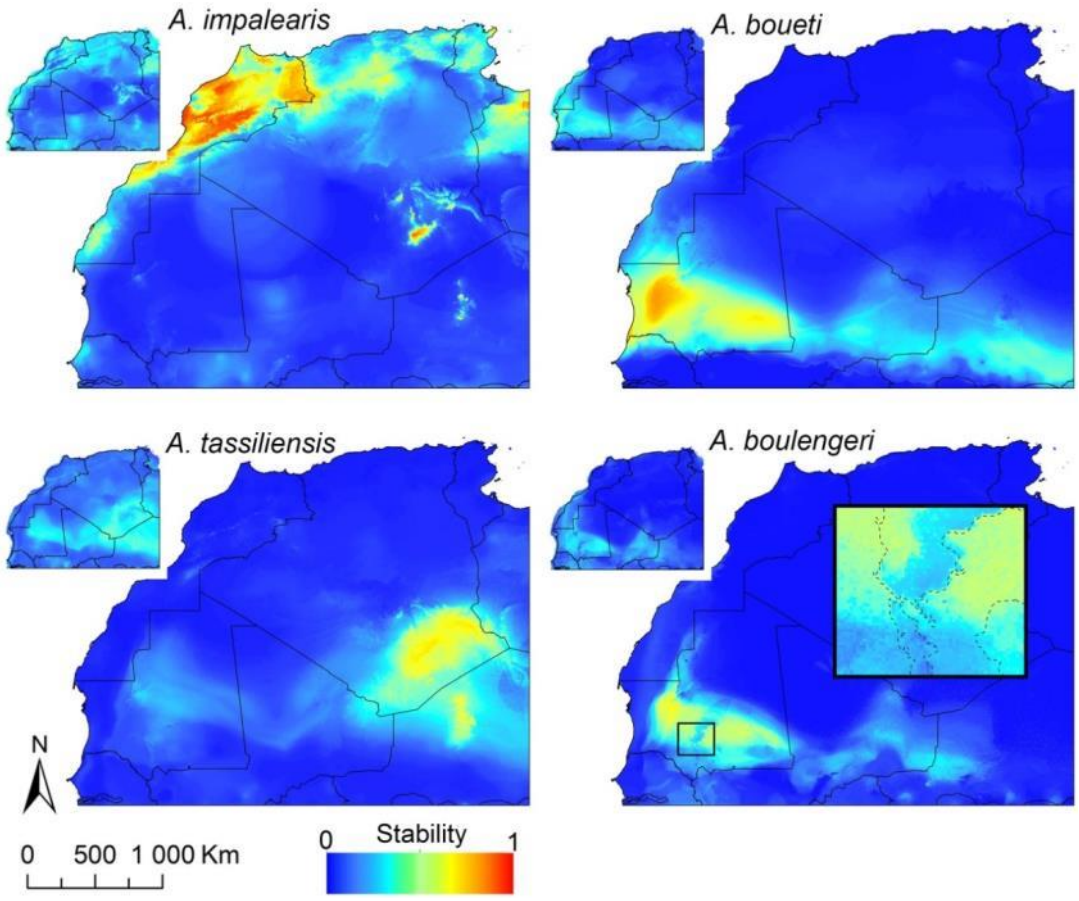

Figure 5. 8. Stable climatic areas for *Agama* species, obtained by averaging the occurrence probability for the Present and the projections for mid-Holocene, LGM and LIG. Warmer colours depict areas with higher stability. Small maps in the lower right corner represent the standard deviation for the maps in the same relative position. Dashed lines in the *A. boulengeri* map inset represent the limits of the Mauritanian mountains.

4.1. Spatial structure of genetic variability

The Agaminae subfamily most likely colonized Africa from the Arabian Peninsula through semi-arid corridors, the Sahel region being among them (Kissling et al., 2016) and, as expected from an Afro-tropical group, most of the Agama diversity in North Africa is found in the Sahel. The geographical coverage of available genetic information and the number of major intra-specific genetic lineages identified have greatly increased from previous studies (Gonçalves et al., 2012; Leaché et al., 2014; Mediannikov et al., 2012). Species crown ages and lineage split times were all younger than the oldest age of the Sahara (7 Ma; Schuster et al., 2006), and mostly fall within the Pliocene-Pleistocene (*A. boulengeri* crown age is 6.4Ma), a pattern shared with other taxa in the region, including reptiles, birds and mammals (Brito et al., 2014), and usually attributed to the aridification and/or climatic cycles.

Species and lineages are almost exclusively parapatric or allopatric, which verifies the first expected pattern under the hypothesis of climate-induced vicariance and allopatric diversification. Aridity-induced isolation in sky islands, a pattern also present for instance in *Myrtus* shrubs (Migliore et al., 2013) or *Ptyodactylus* geckos (Metallinou et al., 2015), is apparent through the one-per-mountain-range distributions of the lineages of *A. tassiliensis* and *A. boulengeri*. Evidence of N-S vicariance along the Atlantic coast is found in the North-South separation of sister species *A. impalearis* and *A. boueti*, in agreement with previous studies (Gonçalves et al., 2012), and matching the generally recognized role of the Sahara in separating Mediterranean and Sahelian populations (Brito et al., 2014; Douady et al., 2003). Still, the role of the Atlantic coast as a corridor allowing species dispersal across the desert (Brito et al., 2014; Gonçalves et al., 2018) is illustrated by *A. impalearis* being the only species north of the Sahara, and the fact that in the present time the distributions of these two species are very close, almost parapatric.

Although genetic analyses revealed no signs of contact between *A. impalearis* and *A. boueti*, the difficulty of sampling in the geographic area between both species prevents ruling out possible secondary contacts. A past secondary contact between *A. boueti* and *A. tassiliensis* in Hoggar mountains, previously hypothesised based on signs of nuclear introgression (Gonçalves et al., 2012), was supported by the palaeo-models (Fig. 5.8, B3). If that was the case, it would contrast with the pattern found in other terrestrial vertebrates like the snake *Psammophis schokari* (Gonçalves et al., 2018) or the anurans *Pelophylax saharicus* (Nicolas et al., 2015) and *Bufotes boulengeri* (Nicolas et al., 2017), which seem to have reached the Hoggar mountains from the

north. The phylogeographic pattern recovered for *A. boulengeri* of deep allopatric mitochondrial lineages with low nuclear diversity and extensively shared haplotypes may indicate climate-mediated range fluctuations and secondary-contact events, but further research is needed to clarify it (Appendix B, Text B1).

4.2. Climatic refugia

The climatically stable areas and areas of predicted present occurrence for species were quite separated, with large extensions of highly unsuitable regions between them (Figs 5.8, B3), suggesting climatic conditions did not favour contact. The general correspondence of climatically stable areas with species and lineage distribution in the impalearis-boueti-tassiliensis group is concordant with the vicariance hypothesis, since they indicate potential refugia (Carnaval et al., 2009) whose geographic isolation can lead to allopatric speciation (Avise, 2000). However, in the particular cases of impalearis NW and SE it is more likely due to a colder and more humid climate in the Atlas Mountains (Figs 5.8, B3), rather than increased aridity, similarly to other taxa in that region like *Discoglossus* frogs, *Mauremys* pond turtles or *Daboia* snakes (Lansari et al., 2014; Veríssimo et al., 2016; Martínez-Freiría et al., 2017). For *A. boulengeri,* the stable climatic areas include sandy areas where the species does not presently occur, which could indicate that only the outskirts of the mountains, rather than the whole massifs, acted as microrefugia not detected by the models, or that there was more exposed rock in the past. It could also be an artefact from a climatic layer dataset that does not include habitat and thus is insufficient to predict the stable areas for a species occurring almost exclusively on rocky outcrops [see Vale et al., (2012) for topoclimatic models for the present].

The other two only other available studies applying multiple-climatic-phase palaeodistribution modelling in north Africa found contrasting patterns: Martínez-Freiría et al. (2017) found a similar pattern of allopatric potential refugia broadly coherent with lineage distributions of *Daboia* snakes in Northern Maghreb, while Gonçalves et al. (2018) found signs of a continuous coastal climatic refugium along the Atlantic for *Psammophis schokari*. Although in the latter case the stable areas were continuous in terms of climate, habitat suitability was likely interrupted by the basin of the large intermittent (flowing in humid phases) Tamanrasset paleo-river, which depicts a different example of the impact of Pleistocene climatic cycles in species diversification in the region. Although not employing paleomodeling, refugia in arid North Africa have been suggested in multiple studies in the last decades. Most of the focus has been on mesic species, with refugia including the mountain systems of central Sahara

Table 5. 5. Niche comparisons at lineage, species, and intra-genus branch levels. *D* = measured niche overlap; Equiv = equivalency test; B -> A and A -> B = similarity tests. Significant ($p<0.05$) similarity tests are marked in grey. Equivalency tests are not directly comparable with those calculated using the old methodology (Broennimann et al., 2012; see discussion and Text B2 in Appendix B).

Level	Comparison (A-B)	*D*	Equiv.	B -> A	A -> B
Lineage	boue_C - boue_E	0.155	0.968	0.082	0.094
	boue_C - boue_W	0.111	1.000	0.082	0.068
	boue_E - boue_W	0.058	0.998	0.236	0.230
	boul_E - boul_N	0.125	1.000	0.046	0.030
	boul_E - boul_S	0.579	0.002	0.002	0.004
	boul_N - boul_S	0.207	0.002	0.006	0.008
Species	boue - boul	0.036	1.000	0.365	0.307
	boue - imp	0.035	1.000	0.419	0.365
	boue - tass	0.032	1.000	0.573	0.561
	boul - imp	0	1.000	1.000	1.000
	boul - tass	0.029	1.000	0.234	0.214
	imp - tass	0.055	1.000	0.305	0.355
Branch	bit* - boul	0.01	1.000	0.070	0.060

* - boueti-impalearis-tassiliensis group

(Metallinou et al., 2015), inland water bodies of large scale like Lake Chad (Granjon and Dobigny, 2003) and micro-scale like oases (Shaibi and Moritz, 2010), or the peripheral regions of Sahel and the Mediterranean (Douady et al., 2003). Refugia for aridity-adapted species, that should have suffered range contractions during wet periods (Carranza et al., 2008; Metallinou et al., 2012; Pook et al., 2009; Tamar et al., 2016), is comparatively lacking. In fact, climatic shifts can be so fast and pronounced (DeMenocal et al., 2000), that many species probably need refugia for both humid and arid periods. That is exemplified by the aridity-adapted reptile genus *Mesalina*, that may survive in the outskirts of the central Saharan mountains in periods of extreme aridity (Kapli et al., 2015). The pattern of refugia is still not well understood in North Africa, but based on examples from other regions, like the reptile community in south-western Australia, the interaction of climate, physical barriers, and range shifts can be quite complex (Edwards et al., 2012). Techniques like demographic simulations could help addressing this subject with more detail and accuracy, as illustrated by a related example about *Lerista lineopunctulata* lizard (He et al., 2013), but proper population sampling and higher number of loci are needed in to pursue such approach.

4.3. Range dynamics

The mountain regions of southern Mauritania seem to have been the origin of dispersal of extant diversity of *A. boueti* and *A. boulengeri*, according to evidence from the diffusion models, interpolation of genetic diversity, and climatic stability. A second, younger possible ancestral area is depicted in Niger for *A. boueti*, which could indicate quick dispersal or a more ancient widespread distribution in the Sahel, but the sampling

gap between Mauritania and Niger preclude further inferences. The diffusion models predicted a recent expansion to the fringes of distribution in Mauritania, a pattern also reflected by lower genetic diversity in those areas (Fig. 5.7). The marked difference in expansion signal (Fig. 5.7; Tables 5.3, B3) recovered in the northernmost lineages of *A. boueti* and *A. boulengeri* (C and N, respectively), when compared to the southern ones from less arid regions, indicates a relatively recent colonization starting from the climatically more stable central-southern areas. The niche similarity among lineages suggests the expansion was not due to adaptation to different ecological conditions, while the fact that "backwards" migrations in the diffusion models were only predicted for recent times (Dryad pending code) suggests repeated local extinctions and loss of signal from previous range expansions. Climatic changes in the region can be sudden and pronounced enough to allow it (DeMenocal et al., 2000). The synchronous marked expansion to the North and East in *A. boulengeri*, and North and West in *A. boueti*, at c.320Ka (Fig. 5.5), followed a glacial termination (Lisiecki and Raymo, 2005), suggesting they took place in a more humid phase. The stable climatic suitability areas around the southern Mauritanian mountains further stresses the importance of opportunity of altitudinal displacement for the survival of mesic species as climate fluctuates (Dobrowski, 2011; Velo-Antón et al., 2013). This pattern of apparent lack of adaptation to fluctuating climate indicates niche conservatism (Kozak and Wiens, 2010), and is another indication of the role of climate in the dispersal and subsequent diversification of mesic species.

4.4 Ecological niche comparisons

Observed niche overlap decreased as phylogenetic distance increased, as expected from a Brownian motion of niche diversification and low degrees of ecological adaptation (Wiens and Graham, 2005). It could happen that niche overlap is biased by geographical proximity (Warren et al., 2008), so overlap alone would not be highly significant since we are comparing two Sahelian lineages. However, the randomized niche similarity tests displayed a similar trend: similarity among species was much lower than among lineages. Given the wide geographical scale, the fact that no dissimilarity test was significant (all $p>0.4$) again shows support for some conservatism of niche and susceptibility to vicariance. The higher similarity among intra-generic branches (*A. boulengeri* vs. the rest) even if not significant, does not contradict the vicariance hypothesis, as the divergence between both groups took place well before the significant increase in aridity (Fig. 5.6), and is also coherent with a general niche conservatism within the genus and subfamily (Kissling et al., 2016).

The significant similarity for all comparisons among lineages of *A. boulengeri* (Tables 5.5, B5) also follows the expected pattern under the hypothesis of vicariance. The *A. boueti* lineages C and E follow the same pattern, but it is clear that boueti W has a less similar climatic niche compared to the rest. Given no allele sharing in NTF3, this could indicate some level of adaptation or divergence, and is compatible with the existence of an undescribed species and the proposal of a "boueti" species complex by other authors based on the morphological and ecological variation within *A. boueti* (Leaché et al., 2014; Mediannikov et al., 2012).

It is worth noting that niche distinction between *A. boueti* and *A. impalearis* is much greater than among *A. boulengeri* lineages of around the same age. Together with the case of boueti W, this could indicate a lower degree of niche conservatism in the boueti-impalearis-tassiliensis group, which could translate into higher adaptation potential to different climates and habitat (Wiens et al., 2010), and help explain why only one of the groups was able to cross and colonize the Sahara. However, the rock habitat specialization of *A. boulengeri* is the most likely cause for its geographically restricted occurrence; species of the boueti-impalearis-tassiliensis occur in soft to hard soil with sparse vegetation, and therefore do not require rock connectivity to disperse to different areas.

Equivalency tests were mostly non-significant, meaning the areas where the compared entities occur are more different than expected by chance (except among *A. boulengeri* lineages). This can be explained by all of the compared areas being allopatric, and in general having low ecological niche overlap. Plus, equivalency tests are not weighted by the distribution density, meaning they include all the area within the minimum convex polygon without correcting for actual occupancy (like the similarity tests and niche overlap measurement do); and given the characteristics of the landscape, a significant portion of the area within the minimum convex polygons can actually be unsuitable for both entities, possibly rendering comparisons meaningless.

Previous studies have reported low p-values for equivalency tests (Ahmadzadeh et al., 2016; Gonçalves et al., 2018; Martínez-Freiría et al., 2017; Rato et al., 2015), but the ones obtained here cannot be directly compared to those results, given differences in the way the p-value for equivalency tests is calculated in ECOSPAT and in previous scripts (Broennimann et al., 2012). If subject to the previous methodology the equivalency values here obtained would be close to 0, thus following the same pattern as other published works (Appendix B, Text B2).

Most studies addressing niche divergence and conservatism in North Africa are focused in the Mediterranean region (e.g. Anadón et al., 2015; Rato et al., 2015), with examples relating varying levels of niche divergence to patterns of temperature seasonality in Mediterranean and Atlantic climates (Ahmadzadeh et al., 2016), or linking niche conservatism and allopatric diversification to Pleistocene climatic oscillations (Martínez-Freiría et al., 2017). Diversification in a scenario of climatic niche conservatism has been described for other arid regions as well (e.g. Loera et al., 2012), and it might be a common pattern. These examples show that adaptation or niche specialisation are not requirements for diversification in deserts. A similar conclusion have been reached by Wiens et al. (2013) when comparing niche breaths of Phrynosomatid lizards, and concluding that high diversity in arid regions was likely due to longer time evolving in those habitats.

5. Conclusions

This is, to our knowledge, the first study evaluating the role of aridity-induced vicariance in species diversification across the Sahara-Sahel ecoregions. Using *Agama* species as a model, we have verified the occurrence of the expected patterns for species with a conserved niche under a climatically-induced vicariance scenario. However, fine-scale assessments of gene flow, especially in the putative contact-zones, evaluating the role of the landscape, and increased genetic datasets that allow modelling demographic scenarios are still needed to fully understand the evolution of this group and by extension other species in the region.

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5.3. Article V. Phylogeography of *Hoplobatrachus occipitalis* in Mauritania provides another piece to the diploid-tetraploid frog puzzle?

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Abstract

Numerous geological and climatic events have affected evolutionary patterns of the biological diversity of North Africa and the Sahara-Sahel in the last few million years. Although several studies have assessed the phylogeographic patterns of vertebrates, relatively few are available for amphibians. The particular water requirements, intermediate between other non-volant vertebrates like reptiles/mammals and fish, make them of particular interest for evolutionary studies. Here we aimed at assessing the evolutionary processes affecting diversification of water-dependent species in arid North Africa, using Mauritanian populations of the widespread tiger frog *Hoplobatrachus occipitalis* as a study subject. Using mitochondrial and nuclear markers, we have assessed the species' genetic structure, distribution of genetic diversity, and the presence of cryptic diversity. We found evidences that support the hypothesis of a recent (re-)colonization of the mountains in its northernmost distribution, but also for the role of southern Mauritanian mountains as refugia. Two major lineages were detected, one of which may have originated by an allopolyploidy event, which could make *H. occipitalis* one of the few examples in North Africa of diversification in the absence of vicariance or ecological adaptation.

1. Introduction

Numerous geological and climatic events have affected the geographic and biological diversity of North Africa in the last few million years (Fabre, 2005; Le Houérou, 1997). Climatically, the most wide-ranging event was a shift from tropical to arid environments around mid-Miocene (Zachos et al., 2001) that eventually led to the appearance of the Sahara desert between 7 million years ago (Ma) and 2.5 Ma (Schuster et al., 2006; Swezey, 2009; Zhang et al., 2014). Arid and humid conditions then alternated during the last few million years, causing a series of expansions and contractions of climatic zones (Le Houérou, 1992; Swezey, 2009) that largely determined current biodiversity patterns (Brito et al., 2014).

Several mechanisms of diversification have been suggested, including genome rearrangements (Dobigny et al., 2005), adaptation to novel habitats (Boratyński et al., 2012; Carranza et al., 2002; Guillaumet et al., 2008) or, most commonly, divergence in allopatry (Brito et al., 2014). Species range shifts associated to climatic cycles resulted in disjoint distributions and allopatric diversification, observable for example in isolated populations in Saharan highlands (Geniez and Arnold, 2006; Metallinou et al., 2015) or

rock pools (Brito et al., 2011; Vale et al., 2015).

Several studies have assessed the phylogeographic patterns of vertebrates in North Africa and Sahara-Sahel, but relatively few are available for amphibians (Brito et al., 2014). Amphibians in general are considered poor dispersers, usually leading to a high spatial genetic structure (Zeisset and Beebee, 2008), thus being good candidates for inferring biogeographic scenarios. Also, amphibians are of particular interest for studying the evolutionary impact of climatic fluctuations in arid regions because they present quite different water requirements than other non-volant animals, like mammals or reptiles, and therefore have different distribution and dispersal constraints. Although all species require water for reproduction, some need it permanently (e.g. *Hoplobatrachus*, *Pelophylax*), while others more temporarily (e.g. *Sclerophrys*, *Tomopterna*). Also, unlike fish, amphibians are not completely restricted to river beds (at least anurans, the ones occurring in the Sahara-Sahel). Being able to keep gene flow between water courses during rainy seasons should decrease the genetic diversity loss caused by occasional local draughts, thus creating a buffer effect against regional extinctions or genetic drift that would more easily affect for instance the fish fauna. However, it is still unclear how amphibians are affected by the climatic cycles.

The few studies available have revealed distinct phylogeographic patterns. While some species of amphibians from North Africa, such as *Hyla meridionalis, Bufotes boulengeri* or *Discoglossus pictus* present structured genetic diversity (Lansari et al., 2014; Recuero et al., 2007; Stöck et al., 2006; Zangari et al., 2006), others show minimal intraspecific variation, like *Sclerophrys mauritanica* (Harris and Perera, 2009) or *Sclerophrys xeros* (Froufe et al., 2009). Possible relict populations have also been found, as for instance *Pelophylax saharicus* or *Bufotes boulengeri* in the mountains of southern Algeria, likely isolated during the humid-arid cycles in the Pleistocene (Nicolas et al., 2017, 2015). However, most of the phylogeographic knowledge on amphibians in North Africa comes from the Mediterranean region while the southern Sahara and Sahel remains mostly unassessed (Padial et al., 2013).

In this study, we aim to assess the evolutionary patterns and processes of diversification of water-dependent species in arid North Africa, using *Hoplobatrachus* as a study subject. *Hoplobatrachus* is a genus of widespread frogs that originated in Asia, whose only African representative, *H. occipitalis*, resulted from a dispersal event that likely took place in the Miocene (Kosuch et al., 2001). Although it is considered a widespread species, previous studies have identified tetraploid and diploid populations in Liberia, raising the possibility of the existence of cryptic diversity (Bogart and Tandy,

1976). *H. occipitalis* is found throughout Western and Central Africa occurring almost exclusively in permanent water bodies like river banks, rock pool and savannah ponds (Rödel, 2000), dispersing and reproducing only when rainfall is enough to fill the temporary ponds (Spieler and Linsenmair, 1997, 1998). It has been suggested that this species was part of a re-colonisation trend that affected some desert mountains systems during the Last Interglacial, including the Adrar-Atar in Mauritania, or Tibesti and Ennedi in Chad (Dumont, 1982). Taking in to account that such range shifts in response to climatic cycles have been linked to relict populations and cryptic diversity in mountain regions (Brito et al., 2014), and considering the taxonomy of some species currently described as broadly-distributed, including e.g. *Ptychadena* spp. [Ptychadenidae], *Hoplobatrachus occipitalis* [Dicroglossidae], *Tomopterna* spp. [Pyxicephalidae], and *Phrynobatrachus* spp. [Phrynobatrachidae] may be unreliable (Padial et al., 2013), it is possible that studying the genetic structure of populations in topographically heterogeneous regions in the fringes of the Sahara might not only help explain the evolutionary processes at play, but also reveal undescribed diversity. Increasing data on the distribution, genetic diversity and phylogeographic patterns is also a priority for amphibian conservation in the Sahel (Padial et al., 2013).

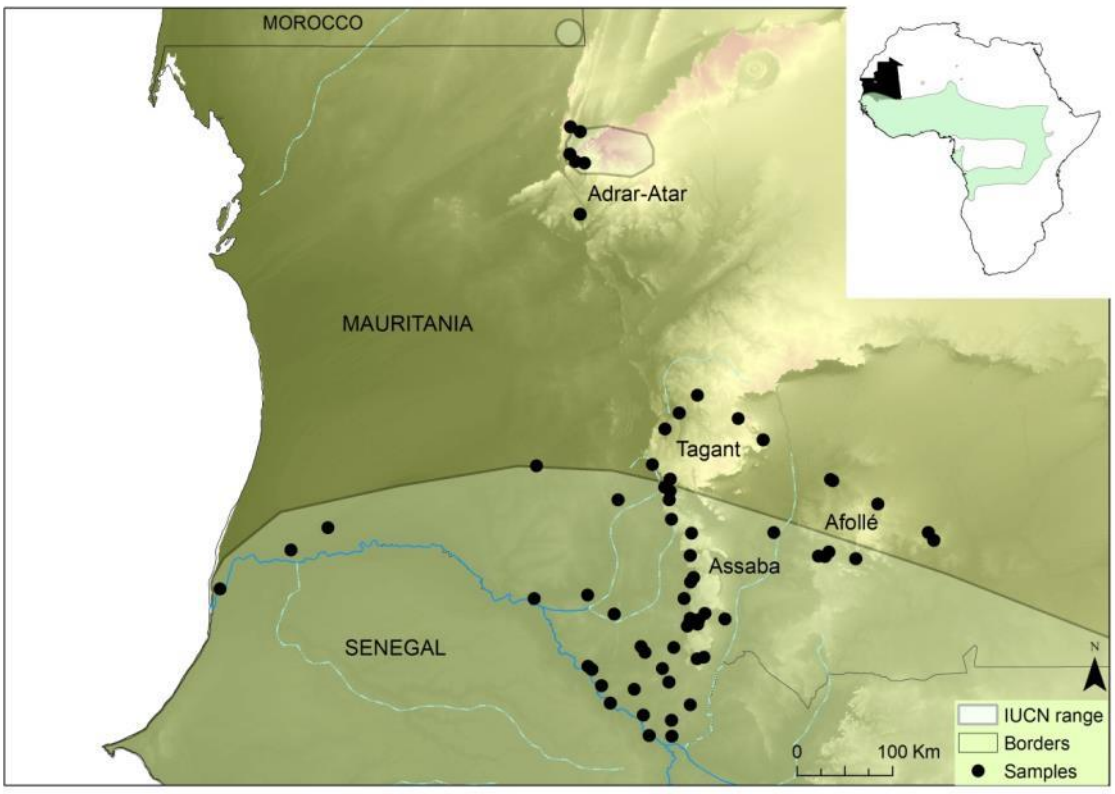

Figure 5. 9. Study area, distribution of *Hoplobatrachus occipitalis* and location of tissue samples used in this study.

Here we propose assessing the spatial genetic structure of *H. occipitalis* in order to better understand the evolutionary processes shaping water-dependent species diversity in the Sahara-Sahel. In order to do that, we will use samples from the specie's range in Mauritania to answer the following questions: 1) How is genetic variability spatially structured?; 2) Where are the areas of higher genetic diversity?; and 3) is there cryptic diversity? Considering this species requires permanent water, we expect the climatic cycles to have had a major impact in the recovered genetic signature. As such, the phylogeographic structure of the species is expected to be related with the hydrographic network. Following the same reasoning, populations in the northern regions and in the lowlands are expected to show less genetic diversity than those in the south and in the mountains.

2. Methods

A total of 276 samples of *H. occipitalis* were available for this study (Fig. 5.9). Given the uneven spatial distribution of samples, an initial selection of samples best representing the geographic distribution of each species was carried out. A total of 143 samples from 69 localities were selected (Table 5.6). DNA was extracted from ethanol-preserved tissue using a commercially available kit (Easy-Spin). Amplifications were performed in 10 uL of 2x MyTaq™ Mix and 0.5 uM each primer. PCR conditions were: pre-denaturation at 95 ºC (15 min); 40 cycles with denaturing at 95 ºC (30 s), annealing range of 48-52 ºC (40 s), and extension at 72 ºC (45 s); and final extension at 60 ºC for 12 minutes. Some samples required minor adjustments to conditions. Three genes were amplified: 16S, NTF3 and c-mos. We used primers from Palumbi et al. (1991), Wiens et al. (2008), Vieites et al. (2007), respectively. PCR products were sequenced using cycle sequencing on an automated sequencer.

Sequences from other Hoplobatrachus species and outgroups were retrieved from Genbank. (Table 5.6). The DNA sequence alignments were created with MAFFT v7 (Katoh and Standley, 2013), with default parameters and the Q-INS-i option. Coding portions (NTF3 and POMC) were translated and no stop codons were found. Independent ML trees were inferred for each marker using RAxML v8.1.21 (Stamatakis, 2014), and no topological incongruences suggesting sample curation errors were found. The most appropriate models of molecular evolution and best-fit partitioning scheme were selected using PARTITIONFINDER v.1.1.1 (Lanfear et al., 2012). Settings were: linked branch lengths, MRBAYESmodels, BIC model selection

criterion, and all partition schemes searched. An initial partition scheme by gene was used.

Mitochondrial and nuclear genes were analysed separately. Phylogenetic trees were inferred based on the 16S sequences, using Bayesian Inference (BI) and Maximum Likelihood (ML) methods implemented, respectively, in MRBAYES v3.2.6 (Ronquist et al., 2012) and RAXML v8.1.21 through RAXMLGUI 1.5b1 (Silvestro and Michalak, 2012). The substitution model was applied according to jModeltest 2 (Darriba et al., 2012). We ran two independent chains in MRBAYES. Burn-in was determined by assessing ESS values in TRACER v1.6 (Rambaut et al., 2014), and checking if the PSRF convergence diagnostic was 1 for every parameter in MRBAYES. Runs were combined and a tree generated using MRBAYES. RAXML used a GTR+G model of sequence evolution as per author's instructions, and was set to perform 10 ML searches and 1000 thorough bootstrapping replicates.

Uncorrected *p*-distances within and among species and lineages were calculated in MEGA6 (Tamura et al., 2013) for each mitochondrial marker. Haplotypes for the nuclear sequences of the species of interest were inferred using PHASE 2.1 (Stephens et al., 2001), implemented in DNASP. PHASE ran for 10^4 iterations with a burn-in value of 1000 and a thinning interval of 5. Haplotype networks were produced using TCS V1.21 (Clement et al., 2000) with gaps as missing data and otherwise default parameters. Graphic representations were obtained using tcsBU (Santos et al., 2015).

3. Results

The mitochondrial diversity of *Hoplobatrachus* in Mauritania was clearly divided in two divergent lineages (Fig. 5.10) with a genetic distance of 7 ± 1% (uncorrected p-distance). Their distribution is parapatric, with one lineage being restricted to the mountains of Tagant, Assaba and Afollé in southern Mauritania ("highland" lineage), and the other distributed in Adrar-Atar and the Sahelian lowlands ("lowland" lineage) until Niger (2000km away, outside the map range). Both lineages had little genetic variability, especially the "highland" one, with only two alleles. One of them was only found in a single locality in the southern side of the Djouk valley. Seven alleles were detected in the lowland lineage, two of which in Niger. One was widespread and the rest occurred in Senegal River and the rivers flowing from the western side of Assaba mountains.

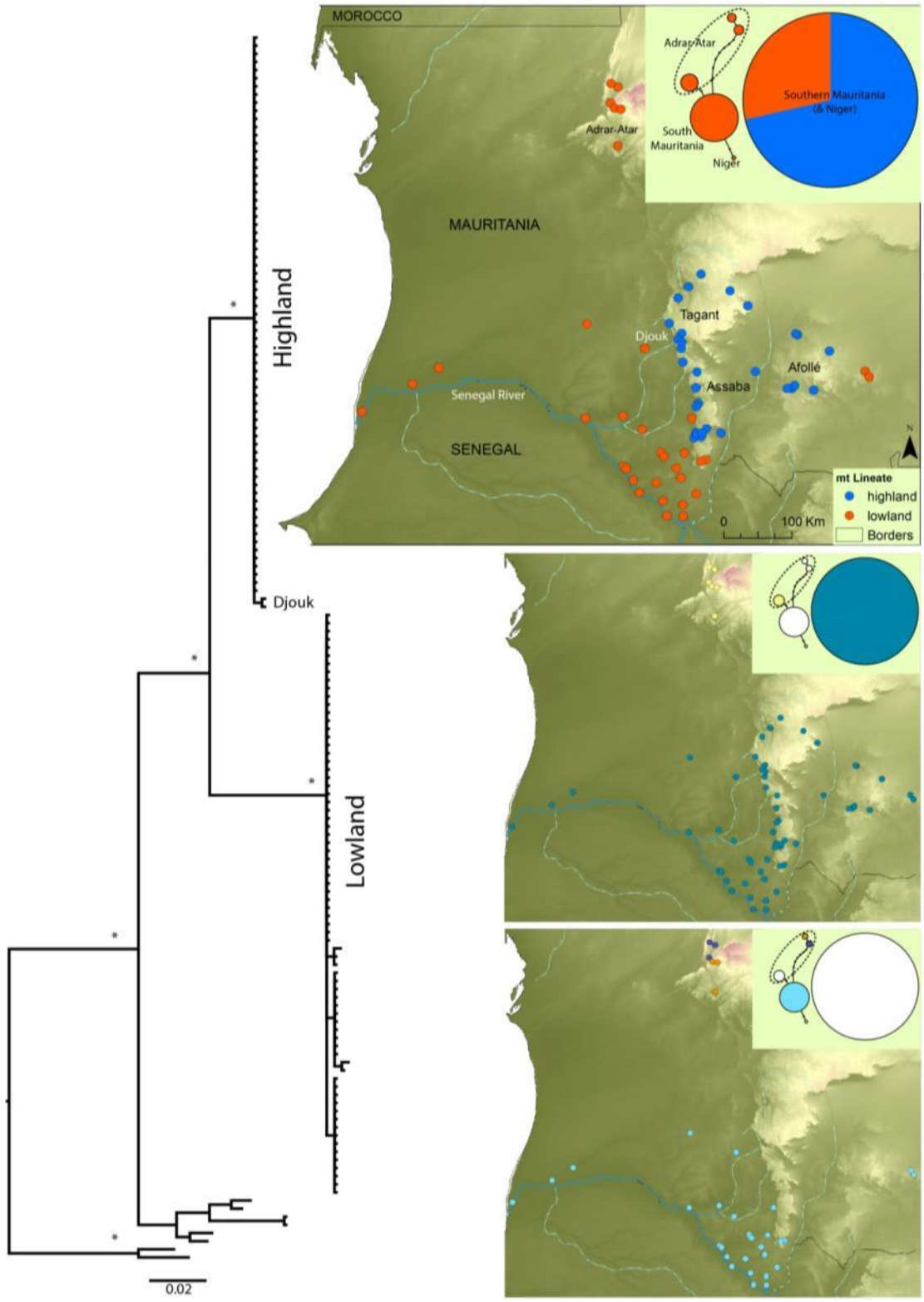

Figure 5. 10. Phylogenetic relationships among *H. occipitalis* populations in Mauritania. **Left:** phylogenetic tree (BI+ML) based on the alignment of 16S sequences; asterisks represent support of 100%pp/100bss; the represented topology is the one obtained with MRBAYES. **Top right:** map depicting the distribution of the recovered mitochondrial lineages, and nuclear haplotype network of POMC coloured according to the phylogenetic tree; each circle denotes one haplotype, and the size is proportional to the number of samples contained. **Centre and bottom right:** maps representing the distribution of each individual nuclear POMC haplotype; each haplotype is represented in only one map; colours in the small haplotype networks denote the represented haplotype.

Nuclear genetic diversity exhibited a very peculiar pattern, with all "highland" samples being homozygotic and having one allele for both nuclear markers, while all "lowland" samples had two alleles and were heterozygotic in all variable positions. In NTF3, only two alleles were recovered in the 49 sequenced samples, three substitutions apart (not represented). In POMC, six alleles were found in 134 samples. Most "lowland" individuals possessed the "highland" allele for both markers, plus a second allele. The Adrar-Atar population shared no nuclear allele with the "highland" populations, but the pattern was similar: all specimens shared a common allele, plus a second one (Fig. 5.10). TCS was not able to connect the POMC highland allele with the others, but based on the sequence alignment it is either 1 or 9 substitutions away Adrar-Atar alleles (and the inverse from the southern lowland allele).

4. Discussion

Here we present one of the first phylogeographic studies on amphibians in the western Sahel (Froufe et al., 2009; Vasconcelos et al., 2010), adding another contribution to the poorly studied region. We have identified two lineages of *H. occipitalis*, whose divergence (7% in 16S) is above sister-species distances reported for the close genus *Fejervarya* (Kotaki et al., 2010), and above the average interspecific distance among the other *Hoplobatrachus* species based on sequences available in GenBank (3.4 – 6.1%). The very low genetic diversity, both for mitochondrial and nuclear markers, is a signal of a very strong and likely recent demographic bottleneck and subsequent expansion. This pattern is similar to what was found in other western Sahelian taxa, for instance in the toad *Sclerophrys xeros* (Froufe et al., 2009) and mammals (e.g. Mouline et al., 2008) with postulated retreats to southern regions (Froufe et al., 2009) or humid refugia (Mouline et al., 2008). Considering these frogs and amphibians in general heavily depend on permanent water, the pattern is likely linked with the aridity cycles (Brito et al., 2014). Similar patterns have also been found in the north-western side of the Sahara, in Morocco, where some amphibians species present low genetic diversity, probably due to similar climatic constraints (Nicolas et al., 2015). The parapatric distribution of both lineages suggests that during the climatic cycles some populations of the "highland" lineage were isolated and persisted in refugia in the southern mountains, while the "lowland" lineage has retracted its range to the south and subsequently recolonized the areas around the southern mountains and the Adrar-Atar during favourable times.

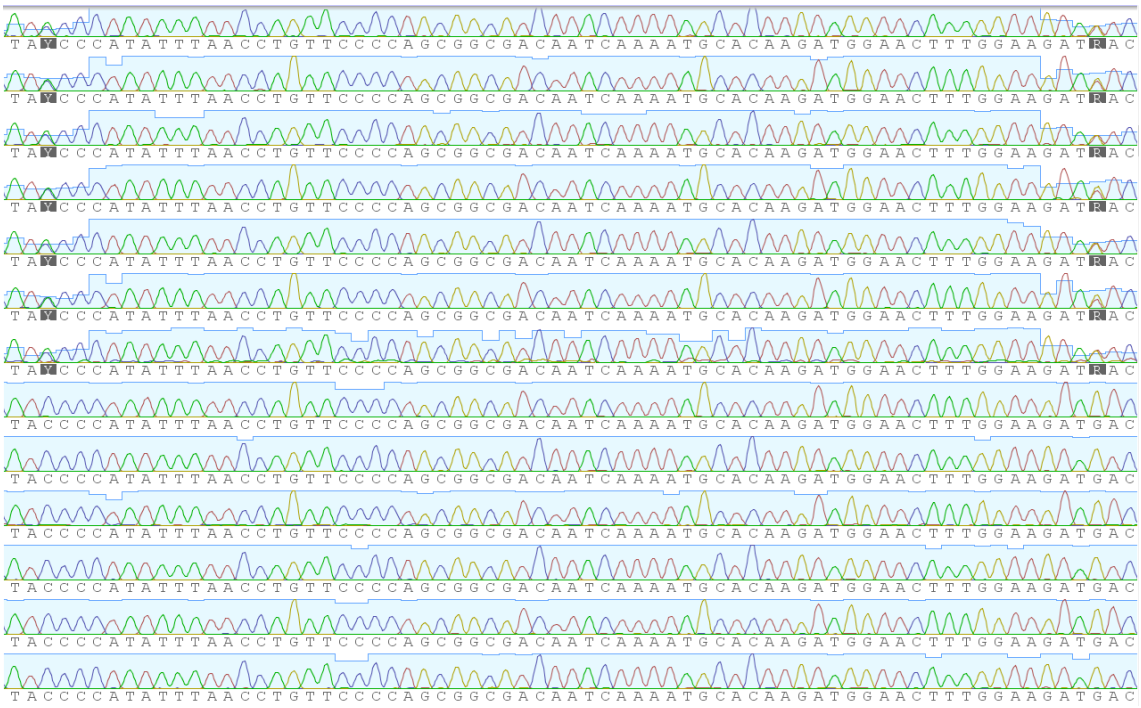

Figure 5. 11. Sections of aligned chromatograms depicting the pattern of heterozygous "lowland" (top 7) and homozygous "highland" specimens (bottom 6).

The locality with the highest "highland" diversity is in the Djouk valley, suggesting a role of that area for species' persistence during arid phases, in line with evidence found in lizards (Gonçalves et al., n.d.), and crocodiles (Velo-Antón et al., 2014). As for the "lowland" lineage, the fact that only one mitochondrial allele is found in western Mauritania, Afollé, and Adrar-Atar is coherent with a recent expansion, but the three nuclear POMC alleles isolated in Adrar-Atar can indicate a refugium. Previous studies with aquatic taxa (fishes and odonates) have proposed that an extended drought during the Holocene led to local extinctions of populations in the Central Saharan Mountains, including Adrar-des-Iforas in Mali-Algeria, Aïr in Niger, Tibesti and Ennedi in Chad, and Adrar-Atar. The later three seem to have been subject to recolonization events during a humid spell later in the Holocene (Dumont, 1982; van Bocxlaer et al., 2011). Relict populations of *H. occipitalis* can still be found in Aïr (IUCN, 2014), one of the mountain systems that are devoid of fish fauna, indicating that they can indeed persist through severe arid periods. Therefore, both persistence and recolonization are likely scenarios even if the species is currently not found in the plains south of Adrar-Atar. Studies in savannah habitats have showed a high dispersal ability, with some animals moving more than 1Km per night during rainy season (Spieler and Linsenmair, 1998). Postures of hundreds of eggs (Alam et al., 2012), and aggressive intra-specific interactions that encourage the occupation of marginal habitats (Spieler and Linsenmair, 1998) probably allows fast recolonizations during favourable climatic phases. The ability of *Hoplobatrachus* to colonize dry environments has been attributed

to tadpole carnivory, which allows an accelerated growth rate essential in ponds prone to quick dissecation (Grosjean et al., 2004). This strategy contrasts with the one found for instance in *Sclerophrys* or *Tomopterna*, that survive by burrowing (Loveridge, 1976). *Sclerophrys xeros* has shown a slightly higher diversity than *H. occipitalis* (Froufe et al., 2009), but whether the differences are linked to life strategies is unclear. The ability of North African amphibians to colonize arid regions is considerable different among species (Padial et al., 2013), so in order to understand the biogeography of the region, the phylogeographic patterns of more species need to be assessed.

The most interesting finding, however, was that all "lowland" specimens were heterozygotic for the nuclear markers. This suggests an event of duplication, which would either have to occur in the regions of both genes, or be evidence of genome duplication. If so, that would be another reason for these two lineages to represent different species. Tetraploid populations of *Hoplobatrachus* have already been described in Liberia, while only diploid ones were found in Cameroon and East Africa (Bogart and Tandy, 1976, 1981). The fact that all heterozygotes belonged to one mitochondrial lineage while all homozygotes belong to other seems to indicate reproductive isolation between both lineages, which would be expected from chromosomal incompatibility. Also, it would suggest an allopolyploid hybrid origin (e.g. *Bufotes turanensis*; Stöck et al., 2006), given the highland and lowland alleles are quite far apart, with no intermediate detected that could be attributed to substitutions after an autopolyploidy event. The fact that most "lowland" specimens possess the "highland" haplotype would suggest that the latter was one of the two ancestor diploid species that hybridized to give rise to the new "lowland" tetraploid species.

While studying post-mating isolation among Dicroglossidae species, Alam and colleagues (2012) have forayed into the possibility of tetraploid formation from hybrid triploids, which they found to be more find than diploid hybrids e.g. in the case of *H. chinensis* X *H. tigerinus*. However, they avoided taking further conclusions and called for further studies on possible polyploidisation and cryptic diversification in *Hoplobatrachus*, *Euphlyctis*, and *Fejervarya* genera. Polyploidy events can happen in nature through several genetic mechanisms, occur repeatedly, and have multiple maternal origins (Schmid et al., 2015; Stöck et al., 2006), and it is an event that is made more likely during periods of climatic instability (Mable, 2011). It is therefore likely than more populations besides Liberia will show the same pattern, but unfortunately no definitive conclusion can be taken regarding the allopolyploidy hypothesis, as no triple or quadruple peak could be found in the nuclear markers. In fact, the only area with more than two alleles is Adrar-Atar, and those alleles are coded in two positions, so it

would actually be impossible to find a polyploid position. Confirmation of polyploidy would be dependent on other techniques, like karyotyping, sequencing faster evolving markers like microsatellites (Shao et al., 2009; Sultana et al., 2014), or using genomic approaches. Another question that remains unanswered is whether the putative tetraploid lineage here suggested belongs to the same species that was previously identified in Liberia. If so, the "highland" *H. occipitalis* could be one ancestral diploid of a widespread tetraploid species, and only one ancestor would remain to be found. If confirmed this would also be the first case of vertebrate diversification by polyploidy in the Sahara-Sahel, and one of the few examples of diversification in the absence of vicariance or ecological adaptation.

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Table 5. 6. List of samples used in this study. Coordinates are in decimal degrees.

SAMPLE	SPECIES	Cluster	16S	NTF3	POMC	LAT	LONG	PROVINCE	COUNTRY
1343	*H. cf. occipitalis*	highland	pending	pending	pending	15.883	-12.036	Assaba	Mauritania
1385	*H. cf. occipitalis*	highland	pending	pending	pending	16.640	-11.056	Assaba	Mauritania
1399	*H. cf. occipitalis*	highland	pending	pending	pending	17.267	-12.199	Tagant	Mauritania
1400	*H. cf. occipitalis*	highland	pending	pending	pending	17.267	-12.199	Tagant	Mauritania
1401	*H. cf. occipitalis*	highland	pending	pending	pending	17.267	-12.199	Tagant	Mauritania
2022	*H. cf. occipitalis*	highland	pending	pending	pending	17.835	-11.558	Tagant	Mauritania
2060	*H. cf. occipitalis*	highland	pending	pending	pending	17.635	-11.324	Assaba	Mauritania
2069	*H. cf. occipitalis*	highland	pending	pending	pending	17.250	-10.668	Hodh El Gharbi	Mauritania
2083	*H. cf. occipitalis*	highland	pending	pending	pending	17.635	-11.324	Assaba	Mauritania
2084	*H. cf. occipitalis*	highland	pending	pending	pending	17.635	-11.324	Assaba	Mauritania
2091	*H. cf. occipitalis*	highland	pending	pending	pending	17.261	-10.690	Hodh El Gharbi	Mauritania

2111	*H. cf. occipitalis*	highland	pending	pending	pending	17.032	-10.245	Hodh El Gharbi	Mauritania
2126	*H. cf. occipitalis*	highland	pending	pending	pending	17.032	-10.245	Hodh El Gharbi	Mauritania
2231	*H. cf. occipitalis*	highland	pending	pending	pending	16.516	-10.453	Hodh El Gharbi	Mauritania
2232	*H. cf. occipitalis*	highland	pending	pending	pending	16.516	-10.453	Hodh El Gharbi	Mauritania
2233	*H. cf. occipitalis*	highland	pending	pending	pending	16.516	-10.453	Hodh El Gharbi	Mauritania
2235	*H. cf. occipitalis*	highland	pending	pending	pending	16.516	-10.453	Hodh El Gharbi	Mauritania
2242	*H. cf. occipitalis*	highland	pending	pending	pending	16.516	-10.453	Hodh El Gharbi	Mauritania
2343	*H. cf. occipitalis*	highland	pending	pending	pending	16.538	-10.742	Assaba	Mauritania
2344	*H. cf. occipitalis*	highland	pending	pending	pending	16.538	-10.742	Assaba	Mauritania
2345	*H. cf. occipitalis*	highland	pending	pending	pending	16.538	-10.742	Assaba	Mauritania
2376	*H. cf. occipitalis*	highland	pending	pending	pending	16.579	-10.705	Assaba	Mauritania
2378	*H. cf. occipitalis*	highland	pending	pending	pending	16.579	-10.705	Assaba	Mauritania
2379	*H. cf. occipitalis*	highland	pending	pending	pending	16.579	-10.705	Assaba	Mauritania
2384	*H. cf. occipitalis*	highland	pending	pending	pending	16.579	-10.705	Assaba	Mauritania
2456	*H. cf. occipitalis*	highland	pending	pending	pending	15.933	-12.011	Guidimaka	Mauritania
2457	*H. cf. occipitalis*	highland	pending	pending	pending	15.933	-12.011	Guidimaka	Mauritania
2458	*H. cf. occipitalis*	highland	pending	pending	pending	15.933	-12.011	Guidimaka	Mauritania
2553	*H. cf. occipitalis*	highland	pending	pending	pending	16.547	-12.010	Assaba	Mauritania
2554	*H. cf. occipitalis*	highland	pending	pending	pending	16.547	-12.010	Assaba	Mauritania
2555	*H. cf. occipitalis*	highland	pending	pending	pending	16.547	-12.010	Assaba	Mauritania
2586	*H. cf. occipitalis*	highland	pending	pending	pending	16.889	-12.185	Assaba	Mauritania
2609	*H. cf. occipitalis*	highland	pending	pending	pending	17.401	-12.364	Tagant	Mauritania
2610	*H. cf. occipitalis*	highland	pending	pending	pending	17.401	-12.364	Tagant	Mauritania
2611	*H. cf. occipitalis*	highland	pending	pending	pending	17.401	-12.364	Tagant	Mauritania
2613	*H. cf. occipitalis*	highland	pending	pending	pending	17.401	-12.364	Tagant	Mauritania
2669	*H. cf. occipitalis*	highland	pending	pending	pending	17.738	-12.245	Tagant	Mauritania
2670	*H. cf. occipitalis*	highland	pending	pending	pending	17.738	-12.245	Tagant	Mauritania
3129	*H. cf. occipitalis*	highland	pending	pending	pending	17.887	-12.111	Tagant	Mauritania
3331	*H. cf. occipitalis*	highland	pending	pending	pending	16.756	-11.997	Assaba	Mauritania
3358	*H. cf. occipitalis*	highland	pending	pending	pending	17.070	-12.208	Assaba	Mauritania
3359	*H. cf. occipitalis*	highland	pending	pending	pending	17.070	-12.208	Assaba	Mauritania
3360	*H. cf. occipitalis*	highland	pending	pending	pending	17.070	-12.208	Assaba	Mauritania
3393	*H. cf. occipitalis*	highland	pending	pending	pending	17.152	-12.199	Assaba	Mauritania
3394	*H. cf. occipitalis*	highland	pending	pending	pending	17.152	-12.199	Assaba	Mauritania
3395	*H. cf. occipitalis*	highland	pending	pending	pending	17.152	-12.199	Assaba	Mauritania
3409	*H. cf. occipitalis*	highland	pending	pending	pending	17.188	-12.248	Assaba	Mauritania
3417	*H. cf. occipitalis*	highland	pending	pending	pending	17.188	-12.248	Assaba	Mauritania
4779	*H. cf. occipitalis*	highland	pending	pending	pending	15.957	-12.010	Guidimaka	Mauritania
4807	*H. cf. occipitalis*	highland	pending	pending	pending	15.901	-11.939	Guidimaka	Mauritania
6017	*H. cf. occipitalis*	highland	pending	pending	pending	17.887	-12.111	Tagant	Mauritania
6018	*H. cf. occipitalis*	highland	pending	pending	pending	17.887	-12.111	Tagant	Mauritania
6036	*H. cf. occipitalis*	highland	pending	pending	pending	18.053	-11.943	Tagant	Mauritania
6086	*H. cf. occipitalis*	highland	pending	pending	pending	16.540	-10.801	Assaba	Mauritania
6087	*H. cf. occipitalis*	highland	pending	pending	pending	16.540	-10.801	Assaba	Mauritania
6088	*H. cf. occipitalis*	highland	pending	pending	pending	16.540	-10.801	Assaba	Mauritania
6113	*H. cf. occipitalis*	highland	pending	pending	pending	17.152	-12.199	Assaba	Mauritania
7694	*H. cf. occipitalis*	highland	pending	pending	pending	16.339	-11.978	Assaba	Mauritania
7706	*H. cf. occipitalis*	highland	pending	pending	pending	16.339	-11.978	Assaba	Mauritania

7707	*H. cf. occipitalis*	highland	pending	pending	pending	16.339	-11.978	Assaba	Mauritania
7719	*H. cf. occipitalis*	highland	pending	pending	pending	16.297	-12.005	Assaba	Mauritania
7720	*H. cf. occipitalis*	highland	pending	pending	pending	16.297	-12.005	Assaba	Mauritania
7724	*H. cf. occipitalis*	highland	pending	pending	pending	15.935	-12.000	Guidimaka	Mauritania
7745	*H. cf. occipitalis*	highland	pending	pending	pending	16.003	-11.872	Assaba	Mauritania
7746	*H. cf. occipitalis*	highland	pending	pending	pending	16.003	-11.872	Assaba	Mauritania
7747	*H. cf. occipitalis*	highland	pending	pending	pending	16.003	-11.872	Assaba	Mauritania
7772	*H. cf. occipitalis*	highland	pending	pending	pending	15.945	-11.929	Guidimaka	Mauritania
7773	*H. cf. occipitalis*	highland	pending	pending	pending	15.945	-11.929	Guidimaka	Mauritania
7774	*H. cf. occipitalis*	highland	pending	pending	pending	15.945	-11.929	Guidimaka	Mauritania
7782	*H. cf. occipitalis*	highland	pending	pending	pending	15.949	-11.682	Guidimaka	Mauritania
7783	*H. cf. occipitalis*	highland	pending	pending	pending	15.949	-11.682	Guidimaka	Mauritania
7867	*H. cf. occipitalis*	highland	pending	pending	pending	16.763	-11.223	Assaba	Mauritania
1315	*H. occipitalis*	lowland	pending	pending	pending	16.142	-13.477	Gorgol	Mauritania
1324	*H. occipitalis*	lowland	pending	pending	pending	15.996	-12.723	Gorgol	Mauritania
1325	*H. occipitalis*	lowland	pending	pending	pending	15.996	-12.723	Gorgol	Mauritania
1644	*H. occipitalis*	lowland	pending	pending	pending	20.323	-13.142	Adrar	Mauritania
1645	*H. occipitalis*	lowland	pending	pending	pending	20.323	-13.142	Adrar	Mauritania
1646	*H. occipitalis*	lowland	pending	pending	pending	20.323	-13.142	Adrar	Mauritania
1668	*H. occipitalis*	lowland	pending	pending	pending	20.534	-13.044	Adrar	Mauritania
1669	*H. occipitalis*	lowland	pending	pending	pending	20.534	-13.044	Adrar	Mauritania
1670	*H. occipitalis*	lowland	pending	pending	pending	20.534	-13.044	Adrar	Mauritania
1714	*H. occipitalis*	lowland	pending	pending	pending	20.581	-13.136	Adrar	Mauritania
1715	*H. occipitalis*	lowland	pending	pending	pending	20.581	-13.136	Adrar	Mauritania
1737	*H. occipitalis*	lowland	pending	pending	pending	20.253	-13.088	Adrar	Mauritania
1738	*H. occipitalis*	lowland	pending	pending	pending	20.253	-13.088	Adrar	Mauritania
1739	*H. occipitalis*	lowland	pending	pending	pending	20.253	-13.088	Adrar	Mauritania
1846	*H. occipitalis*	lowland	pending	pending	pending	19.757	-13.044	Adrar	Mauritania
1847	*H. occipitalis*	lowland	pending	pending	pending	19.757	-13.044	Adrar	Mauritania
1848	*H. occipitalis*	lowland	pending	pending	pending	19.757	-13.044	Adrar	Mauritania
1888	*H. occipitalis*	lowland	pending	pending	pending	20.237	-13.005	Adrar	Mauritania
1889	*H. occipitalis*	lowland	pending	pending	pending	20.237	-13.005	Adrar	Mauritania
1890	*H. occipitalis*	lowland	pending	pending	pending	20.237	-13.005	Adrar	Mauritania
2153	*H. occipitalis*	lowland	pending	pending	pending	16.764	-9.770	Hodh El Gharbi	Mauritania
2154	*H. occipitalis*	lowland	pending	pending	pending	16.764	-9.770	Hodh El Gharbi	Mauritania
2155	*H. occipitalis*	lowland	pending	pending	pending	16.691	-9.717	Hodh El Gharbi	Mauritania
2156	*H. occipitalis*	lowland	pending	pending	pending	16.691	-9.717	Hodh El Gharbi	Mauritania
2157	*H. occipitalis*	lowland	pending	pending	pending	16.691	-9.717	Hodh El Gharbi	Mauritania
2158	*H. occipitalis*	lowland	pending	pending	pending	16.691	-9.717	Hodh El Gharbi	Mauritania
2918	*H. occipitalis*	lowland	pending	pending	pending	19.999	-13.289	Adrar	Mauritania
2919	*H. occipitalis*	lowland	pending	pending	pending	19.999	-13.289	Adrar	Mauritania
4523	*H. occipitalis*	lowland	pending	pending	pending	16.600	-15.765	Trarza	Mauritania
4536	*H. occipitalis*	lowland	pending	pending	pending	16.810	-15.416	Trarza	Mauritania
4656	*H. occipitalis*	lowland	pending	pending	pending	15.507	-12.970	Gorgol	Mauritania
4685	*H. occipitalis*	lowland	pending	pending	pending	15.289	-12.536	Guidimaka	Mauritania
4702	*H. occipitalis*	lowland	pending	pending	pending	15.144	-12.010	Guidimaka	Mauritania
4726	*H. occipitalis*	lowland	pending	pending	pending	15.591	-11.880	Guidimaka	Mauritania
4733	*H. occipitalis*	lowland	pending	pending	pending	15.576	-11.944	Guidimaka	Mauritania

4757	*H. occipitalis*	lowland	pending	pending	pending	15.682	-12.163	Guidimaka	Mauritania
4939	*H. occipitalis*	lowland	pending	pending	pending	17.070	-12.689	Assaba	Mauritania
4943	*H. occipitalis*	lowland	pending	pending	pending	17.070	-12.689	Assaba	Mauritania
4974	*H. occipitalis*	lowland	pending	pending	pending	17.391	-13.456	Brakna	Mauritania
6121	*H. occipitalis*	lowland	pending	pending	pending	16.470	-12.485	Assaba	Mauritania
6148	*H. occipitalis*	lowland	pending	pending	pending	15.692	-12.471	Gorgol	Mauritania
6158	*H. occipitalis*	lowland	pending	pending	pending	15.635	-12.433	Guidimaka	Mauritania
6169	*H. occipitalis*	lowland	pending	pending	pending	15.484	-12.271	Guidimaka	Mauritania
6631	*H. occipitalis*	lowland	pending	pending	pending	13.700	9.530	Zinder	Niger
6784	*H. occipitalis*	lowland	pending	pending	pending	13.966	9.281	Zinder	Niger
6785	*H. occipitalis*	lowland	pending	pending	pending	13.966	9.281	Zinder	Niger
7393	*H. occipitalis*	lowland	pending	pending	pending	16.234	-16.430	Trarza	Mauritania
7457	*H. occipitalis*	lowland	pending	pending	pending	15.476	-12.934	Gorgol	Mauritania
7458	*H. occipitalis*	lowland	pending	pending	pending	15.476	-12.934	Gorgol	Mauritania
7459	*H. occipitalis*	lowland	pending	pending	pending	15.476	-12.934	Gorgol	Mauritania
7486	*H. occipitalis*	lowland	pending	pending	pending	15.322	-12.842	Gorgol	Mauritania
7487	*H. occipitalis*	lowland	pending	pending	pending	15.322	-12.842	Gorgol	Mauritania
7497	*H. occipitalis*	lowland	pending	pending	pending	15.159	-12.761	Gorgol	Mauritania
7498	*H. occipitalis*	lowland	pending	pending	pending	15.159	-12.761	Gorgol	Mauritania
7500	*H. occipitalis*	lowland	pending	pending	pending	15.159	-12.761	Gorgol	Mauritania
7546	*H. occipitalis*	lowland	pending	pending	pending	15.047	-12.450	Guidimaka	Mauritania
7547	*H. occipitalis*	lowland	pending	pending	pending	15.047	-12.450	Guidimaka	Mauritania
7586	*H. occipitalis*	lowland	pending	pending	pending	14.853	-12.396	Guidimaka	Mauritania
7587	*H. occipitalis*	lowland	pending	pending	pending	14.853	-12.396	Guidimaka	Mauritania
7588	*H. occipitalis*	lowland	pending	pending	pending	14.853	-12.396	Guidimaka	Mauritania
7613	*H. occipitalis*	lowland	pending	pending	pending	14.846	-12.182	Guidimaka	Mauritania
7614	*H. occipitalis*	lowland	pending	pending	pending	14.846	-12.182	Guidimaka	Mauritania
7616	*H. occipitalis*	lowland	pending	pending	pending	14.846	-12.182	Guidimaka	Mauritania
7618	*H. occipitalis*	lowland	pending	pending	pending	14.996	-12.184	Guidimaka	Mauritania
7619	*H. occipitalis*	lowland	pending	pending	pending	14.996	-12.184	Guidimaka	Mauritania
7620	*H. occipitalis*	lowland	pending	pending	pending	14.996	-12.184	Guidimaka	Mauritania
7628	*H. occipitalis*	lowland	pending	pending	pending	15.356	-12.210	Guidimaka	Mauritania
7629	*H. occipitalis*	lowland	pending	pending	pending	15.356	-12.210	Guidimaka	Mauritania
7630	*H. occipitalis*	lowland	pending	pending	pending	15.356	-12.210	Guidimaka	Mauritania
7674	*H. occipitalis*	lowland	pending	pending	pending	16.143	-12.066	Assaba	Mauritania
7675	*H. occipitalis*	lowland	pending	pending	pending	16.143	-12.066	Assaba	Mauritania

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Chapter 6

Fine scale biogeography

6.1. Niche conservatism, male-biased dispersal and aridification as drivers for deep lineage allopatry and admixture in a desert reptile (*Agama boulengeri*)

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Abstract

Although biogeography research in Sahara-Sahel has increase in recent years, studies about biodiversity patterns and evolutionary processes at fine spatial and temporal scales are almost non-existent. The geo-climatic dynamics that took place in North Africa, particularly the formation of the Sahara and the subsequent humid-arid cycles, have been correlated with several diversification and speciation events, but almost exclusively at large scales. Here we propose to use spatial analysis and complement mitochondrial and nuclear sequence data with rapidly-evolving nuclear microsatellite markers to assess the fine-scale population structure of the rock-dwelling lizard *A. boulengeri*, a non-volant terrestrial vertebrate endemic of the West Sahara-Sahel. Two clusters were identified based on the microsatellite markers, with an East-West structuration, with gene flow. Each cluster roughly contains two out of the four mitochondrial lineages. Microsatellite genetic structure was more related with spatial availability of rock and sand than the evolutionary relationships among mitochondrial lineages, indicating a strong influence of aridification in the species' history and distribution. Ecological models recovered a strong correlation between the occurrence of *A. boulengeri* and rocky outcrops. In the proposed biogeographic scenario, rock-dwelling *A. boulengeri* suffered population bottlenecks due to climatic fluctuations that led to the decrease of nuclear genetic diversity. Topographic heterogeneity allowed survival of the mitochondrial lineages in four neighbouring refugia, which due to male-based dispersal resulted in a parapatric distribution of mitochondrial lineages. We expect the proposed biogeographic scenario to work as a model for proposing and testing evolutionary patterns found in other mesic and/or rock-dwelling species likely affected by the vicariance effect of aridity in the Sahara-Sahel.

Keywords: *Agama boulengeri*, diversification, mito-nuclear discordance, MBD, microsatellites, Sahara-Sahel.

1. Introduction

Biogeography research in Sahara-Sahel has suffered a marked increase in recent years (Brito et al., 2014). However, phylogeographic studies are still mostly developed at large temporal and spatial scales, using mtDNA and slow-evolving nuclear markers to uncover cryptic diversity and study the large-scale distribution of genetic variability of a few species. There is an almost total gap in knowledge of finer-scale spatial and temporal dynamics. Rare exceptions exist, with some studies employing fast-evolving

nuclear markers in crocodiles (Velo-Antón et al., 2014), bees (Franck et al., 2001), plants (Migliore et al., 2013); but the number is much lower than even in other parts of Africa (a Web of Science search for “microsatellite Africa” returned 1636 hits, against 31 for Sahara and 16 for Sahel). For instance, no such studies exist focusing on the evolutionary history of terrestrial vertebrates in the Sahara-Sahel. The closest examples are developed but yet unexplored microsatellite markers (Lopes et al., 2015), studies oriented to conservation (e.g. Silva et al., 2014) or centred in other or larger areas but including some samples from the region (e.g. Leite et al., 2015). Consequently, the processes that shape the patterns of diversity, especially at local scales, are still not well understood. This is mostly due to the lack of samples resulting from the region’s inaccessibility and political instability (Brito et al., 2014) and it is in sharp contrast with other desert regions, like Australia or North America, where many more samples and distribution data are available (e.g. GBIF.org), biogeographic history and evolutionary processes are better studied (e.g. Riddle & Hafner, 2006; Byrne et al., 2008; Pepper et al., 2011), landscape genetics studies are available (e.g. Hagerty et al., 2011), and the application of genetics to conservation has been a concern for many years (e.g. Meffe & Vrijenhoek, 1988).

The lizard genus *Agama* has several species present in the Sahara-Sahel, and their diversification has been mostly attributed to climate-induced vicariance (Gonçalves et al., 2012). North Africa has suffered a progressive aridification process since the mid-Miocene (Zachos et al., 2001) that culminated with the appearance of the Sahara at about 7Ma (Zhang et al., 2014), and was followed by humid-arid cycles in the Pliocene-Pleistocene (Le Houérou, 1997). Taxa diversification in the region has been frequently linked to these climatic fluctuations, mostly through adaptation to new habitats or vicariance (reviewed in Brito et al., 2014). *Agama* species in the region belong to two main branches of the genus, one of which contains only *A. boulengeri*, likely the sister taxon of all other *Agama* (Leaché et al., 2014). *Agama boulengeri* presents deep mitochondrial lineages that share a most recent common ancestor (MRCA) possibly pre-dating the Pliocene-Pleistocene climatic fluctuations. Significant niche conservatism among these old lineages suggests lack of adaptation to different climatic conditions (Gonçalves et al., *in prep*), suggesting that *A. boulengeri* is a good model to study gene-flow and diversification in a scenario of vicariance without macro-ecological adaptation.

This common lizard occurs mostly in rocky outcrops in the mountains of Mauritania and neighbouring regions of Mali (Vale et al., 2012), a fringe area between the Sahara and the Sahel. Empirical field observations indicate that *A. boulengeri* is a rock specialist,

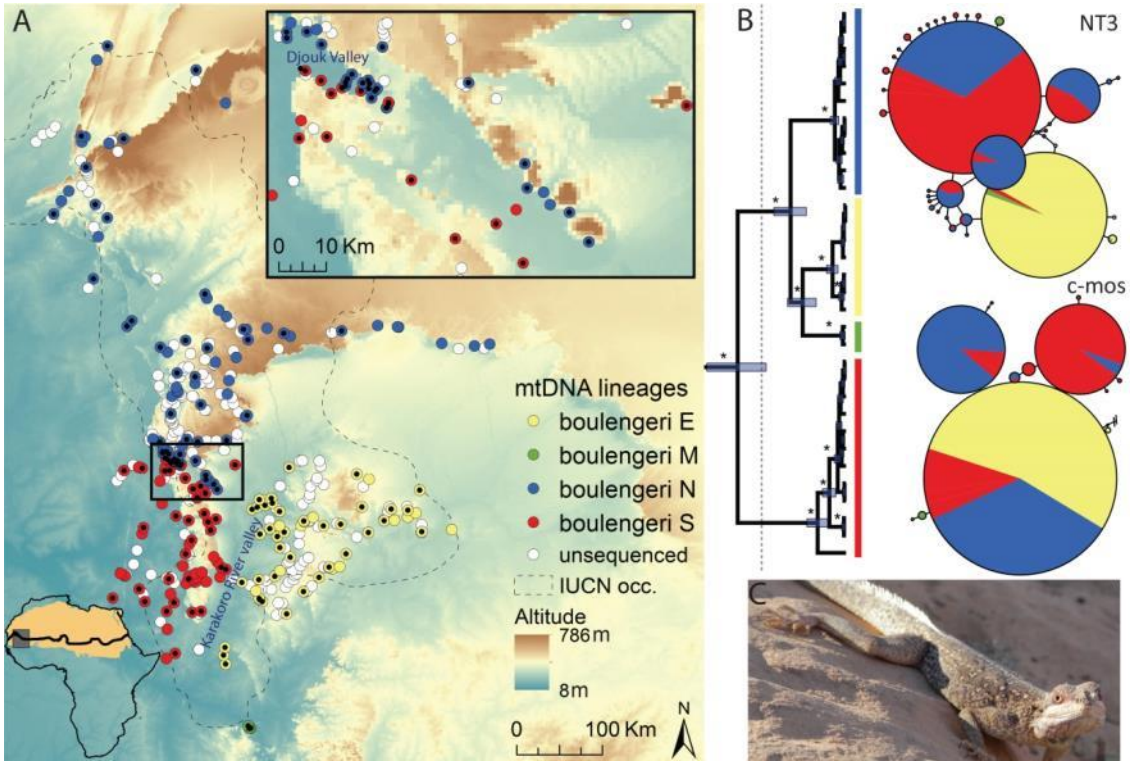

Figure 6. 1. Study area, sample distribution, and genetic structure of *Agama boulengeri*. A) Map of the study area depicting the location of samples; colours indicate the mitochondrial lineage, black dots the genotyped samples. B) Phylogenetic tree (concatenated sequences) and nuclear haplotype networks, adapted from Gonçalves et al. (in prep); asterisks in the phylogenetic tree representing 100% support (Bayesian inference + Maximum likelihood), and the dashed line represents 5 Ma; each circle represents a different haplotype, with size proportional to the number of samples sharing that haplotype. C) Adult male of *A. boulengeri*.

although a previous study using ecological niche models did not find a clear association with rocky habitat (Vale et al., 2012). Previous studies have described four parapatric mitochondrial lineages occupying four neighbouring mountain ranges, separated by more sandy and likely less suitable extensions like the Djouk and Karakoro valleys (Fig. 6.1; Gonçalves et al. 2012; *in prep*). Nuclear genes were not informative regarding the possibility of the existence of cryptic species (Gonçalves et al., *in prep.*), but the clear borders among lineage distributions, even when the putative barriers/filters are quite narrow (particularly the Djouk valley) and the occurrence of some specimens in those valleys, raise the possibility of reproductive isolation.

Here we propose complementing mitochondrial and nuclear sequence data with rapidly-evolving nuclear microsatellite markers to assess the fine-scale population structure of *A. boulengeri*. To our knowledge, this is the first Sahara-Sahel terrestrial vertebrate studied with high-resolution nuclear markers (here microsatellites) so far, and could be a model for the vicariance end of the vicariance-adaptation dynamic shaping biodiversity and evolution in the Sahara-Sahel, expected for mesic species displaying niche conservatism. By integrating fine-scale genetic markers with the ecological and sequence genetic data, we aim to: 1) clarify how genetic variability and

populations are structured; 2) identify the contact zones among intra-specific groups and determine if gene-flow occurs; and 3) demonstrate the strong relationship between rock availability and the presence of *A. boulengeri*. If there is reproductive isolation among lineages, populational structure should follow mitochondrial genetic structure. Considering the parapatric lineage distribution and the amount of time since the MRCA, we expect to find reduced gene flow among mitochondrial lineages. Admitting an influence of climate in lineage distribution and evolutionary processes, based on the apparent lack of niche differentiation, genetic structure is also expected to reflect the habitat suitability and landscape connectivity, and genetic diversity should decrease in areas with more unstable climate due to range fluctuations.

2. Methods

2.1. Genetic analyses

A total of 190 samples covering the complete known distribution of the species were used in this study (Fig. 6.1). Seventeen microsatellite loci were selected based on Gonçalves et al. (2016), by excluding the markers that presented a consistent signal of null alleles. DNA extraction, amplification, sequencing and scoring were performed according to Gonçalves et al. (2016). Observed heterozygosity (H_O), expected heterozygosity (H_E), number of effective alleles (Na) and allele frequencies per population (Fa) were calculated using GenAlEx v6.501 (Peakall & Smouse, 2012), dividing the dataset into groups according to the corresponding mitochondrial lineage (Fig. 6.1). Although this delimitation results in very large geographical scales, the fact that usually only one sample was available per sampling site precluded grouping samples into smaller and more meaningful interbreeding populations.

Table 6. 1. Uncorrected genetic distances (*p*-distance, %) among *Agama boulengeri* mitochondrial lineages. Values were calculated in MEGA 6, using ND4 and 16S mitochondrial sequence data from Gonçalves et al. (in prep). Mali (M) lineage was omitted due to low sample size.

		mt-E	mt-N	mt-S
ND4	E	1.1 ± 0.2		
	N	6.8 ± 0.7	0.8 ± 0.2	
	S	11.5 ± 1	11.7 ± 1	1 ± 0.1
16S	E	0.2 ± 0.1		
	N	4.4 ± 0.9	0.4 ± 0.1	
	S	6 ± 1	6 ± 1	0.4 ± 0.2

Population structure was assessed with the Bayesian clustering approach implemented in STRUCTURE v.2.3.4 (Pritchard et al., 2000). An admixture model without prior population information for individuals and correlated allele frequencies was implemented. We ran 3 independent runs for K=1–7 using all samples with missing data below 25% and an admixture model with correlated allele frequencies. No prior information of sample population membership was provided. Each run consisted of $2x10^5$ burn-in steps, followed by another 8×10^5 steps. Selection of the most likely number of genetic clusters (K) was based on Evanno's method (Evanno et al., 2005) implemented in Structure Harvester (Earl & vonHoldt, 2012). Since only two clusters were found (see results), we used NewHybrids to produce a prediction of the "hybrids" between the two putative parental forms. This was not meant to assume the admixed individuals were resultant of a secondary contact between distinct units rather than an incipient diversification, but to obtain a prediction besides simple thresholds in STRUCTURE cluster assignment. NewHybrids was run with default genotype frequency classes and without prior information regarding individual assignment to a class, and no allelic frequency. We used the Jeffreys-like prior for mixing proportions (Pi and Theta). We used $2x10^5$ burn-in sweeps, followed by 8×10^5 sweeps.

A Principal Components Ordination Analysis (PCoA) was used to obtain a clustering algorithm-free bi-dimensional visual representation of the distance among individuals. We used GenAlEx v6.501 (Peakall & Smouse, 2012) to calculate genetic distances between samples and generate the PCoA.

Spatial explicit representations of genetic diversity were produced using a predefined radius-search around a sample in order to create a pseudo-population from which it estimates diversity. The method was as described by Veríssimo et al. (2016), with minor adaptations to make use of microsatellite heterozygosity as a measure of diversity. The resulting diversity scores can then be spatially interpolated, in this case using the Kriging function in ArcMap (ESRI, 2014). Ten pseudo-populations were created using different sets of samples equally distributed throughout the study area, and interpolation maps were then averaged. We used two radiuses: ~45km, in order to avoid points from two mitochondrial lineages falling inside the same buffer; and ~100km, in order to include the isolated samples that would otherwise be ignored for having no neighbours.

Table 6. 2. Summary statistics for the 17 microsatellite loci, with populations defined according to mitochondrial lineages. N = number of haplotypes; Na = number of different alleles (freq ≥ 5%); Nea = number of effective alleles; H_O = Observed heterozygosity; H_E = unbiased expected heterozygosity; µHe = expected heterozygosity.

	N	Na	Nea	Ho	He	µHe
mt-E	39	10.882	5.983	0.466	0.639	0.65
mt-N	57	13.294	7.12	0.618	0.789	0.797
mt-S	41	12	7.471	0.575	0.795	0.806

2.2. Ecological models

For the ecological niche-based models we had 542 observations covering the full distribution of *A. boulengeri*, with ≤ 1Km resolution (WGS 1984 datum). The study area was defined with a 50Km buffer around a minimum convex polygon including all observations. Spatial analyses were conducted in ESRI ArcGIS 10. In order to reduce spatial bias in the ecological models due to uneven sampling (Merow et al., 2013), localities were removed at random from clusters of species occurrence; the nearest-neighbour index was used to assess the clustering of points. The final dataset included 124 presence points.

Table 6. 3. Climatic variables used to develop ecological niche-based models, and their average (and standard deviation) contribution to the model. LC (land cover) variation of variables is expressed in distance to the nearest pixel of that category.

Code	Name	Units	Variation	Contribution
LC_10	Bare rock	◦	0 - 2.27	38.24 (8.79)
TRI	Terrain Roughness Index	--	0 - 302.75	19.73 (5.08)
LC_3	Compact soil	◦	0 - 1.10	19.17 (6.3)
BIO_12	Annual Precipitation	mm	26 - 901	6.78 (3.21)
LC_4	Croplands	◦	0 - 4.08	3.37 (2.04)
LC_8	Gravel floodplains	◦	0 - 1.56	2.36 (1.67)
LC_14	Rocky soil	◦	0 - 1.53	2.32 (1.05)
BIO_2	Mean Diurnal Range	◦C	131 - 165	2.29 (1.46)
LC_1	Yellow dunes	◦	0 - 1.96	1.62 (1.29)
BIO_5	Maximum Temperature of Warmest Month	◦C	366 - 457	1.52 (1.17)
BIO_1	Annual Mean Temperature	◦C	247 - 301	1.31 (0.84)
BIO_11	Mean Temperature of Coldest Quarter	◦C	179 - 273	1.29 (1.07)

Nineteen variables representing present climatic conditions were downloaded from WorldClim (www.worldclim.org; Hijmans et al., 2005) at 30 arc-second resolution (~1x1 km at equator). After removing highly correlated variables (Band Collection Statistics, threshold of R ≥0.75), and those with clear spatial artefacts, five were kept (Table 6.3). Given that previous modelling efforts failed to detect the relationship between rocky terrain and *A. boulengeri* occurrence, we used new high-resolution land-cover variables that better represent the local landscape (Campos & Brito, *in prep*). Eighteen fine-scale (30m) land-cover categories were converted to continuous variables by calculating the distance of each pixel to the nearest pixel of each category. These were then upscaled to 30 arc-second resolution and subjected to the same procedure as above, resulting in a final dataset of seven variables (Table 6.3). A topographical grid (USGS, 2006) was used to derive a topographic ruggedness index (TRI) using the package "Raster" in R (Hijmans et al., 2016).

Models were computed using the Maximum Entropy approach implemented in Maxent v.3.3.3 (Phillips et al., 2006). The technique requires only presence data as input, and consistently performed well in comparison to other methods (Elith et al., 2006; Hernandez et al., 2006). Models were run with 25 replicates, random seed, 75%/25% training/testing partition, bootstrap with replacement, and auto-features. The area-under-the-curve (AUC) of the receiver-operating characteristics (ROC) plots was used to evaluate individual model fit (Fielding & Bell, 1997). The importance of each variable for predicting the species' distribution was measured by the mean percentage contribution.

2.3. Effect of landscape features in gene-flow

To test the prediction that *A. boulengeri* disperses more easily through rock-connected habitat, we measured the correlation between genetic distances and a set of geographic and landscape resistance distances. Genetic distances among individuals were calculated in GENALEX V6.5. Euclidean, Least Cost Path (LCP), and random walk commute distance (Fouss et al., 2007) were calculated using the R package GDISTANCE (van Etten, 2017). LCP and random-walk distances were calculated based on several classifications of land-cover variables (Appendix C, Table C2). Correlation between matrixes was measured using the Mantel test function implemented in the VEGAN R package (Oksanen et al., 2017), with Pearson method and 9999 permutations.

3. Results

Out of the initial dataset of 190 samples, 140 with missing data in allele sequencing below 25% were kept for further analyses. Alleles per locus varied between 4 and 28 (average 16). Observed heterozygosity (Table 6.2) varied between 0.373 and 0.618 (mean 0.508) and unbiased expected heterozygosity varied between 0.459 and 0.806 (mean 0.678). According to Evanno's method, two (K=2) is the most likely number of clusters (Fig. 6.2; Appendix C, Fig. C1); these are hereafter referred to as μ-W and μ-E, following their relative distribution. Clusters μ-W and μ-E are apparently admixing and roughly include the samples from mitochondrial lineages N+S and E+M, respectively, thus not reflecting their phylogenetic relationship, while revealing a considerable (even more in light of the involved time-scales) mito-nuclear discordance. Analysing μ-E separately revealed some degree of separation between Mali samples and the rest of μ-E, but no differentiation was found in μ-W between the populations of Adrar-Atar, Tagant and Assaba mountains. Using a STRUCTURE cluster assignment threshold of 0.8, the admixed individuals are mostly located in the Karakoro valley. The NewHybrids analysis classified more specimens as admixed, being distributed more broadly throughout most of the southern distribution of the species (Fig. 6.2). Removing these admixed individuals returns a FST among populations of 0.17. The PCoA corroborates the information obtained with Structure, with samples divided between two groups, each including the N+S and E+M mitochondrial lineages (Fig. C2).

Diversity maps show the highest values are located in the centre of the species' distribution, decreasing towards north, east, and south, both in the case of mitochondrial and microsatellite markers. Both buffer radiuses resulted in the same pattern, with the larger one showing an expectable smoothing effect.

Ecological models had a good fit, with mean training AUC of 0.946 (SD 0.006) and test AUC of 0.895 (SD 0.019). Bare rock (large rocky outcrops) was the strongest predictor of the presence of *A. boulengeri*, with the probability of occurrence increasing with the proximity to rock (Table 6.3). The correlation of genetic and Euclidean distances was significant ($p>0.0001$), with an $R=0.22$ (Table C2). The correlation of landscape resistance distance (LCP and random walk) were similar but no consistent improvement was found for classification schemes giving preference to rocky outcrops and soil ($R=0.17$-0.25; Table C2).

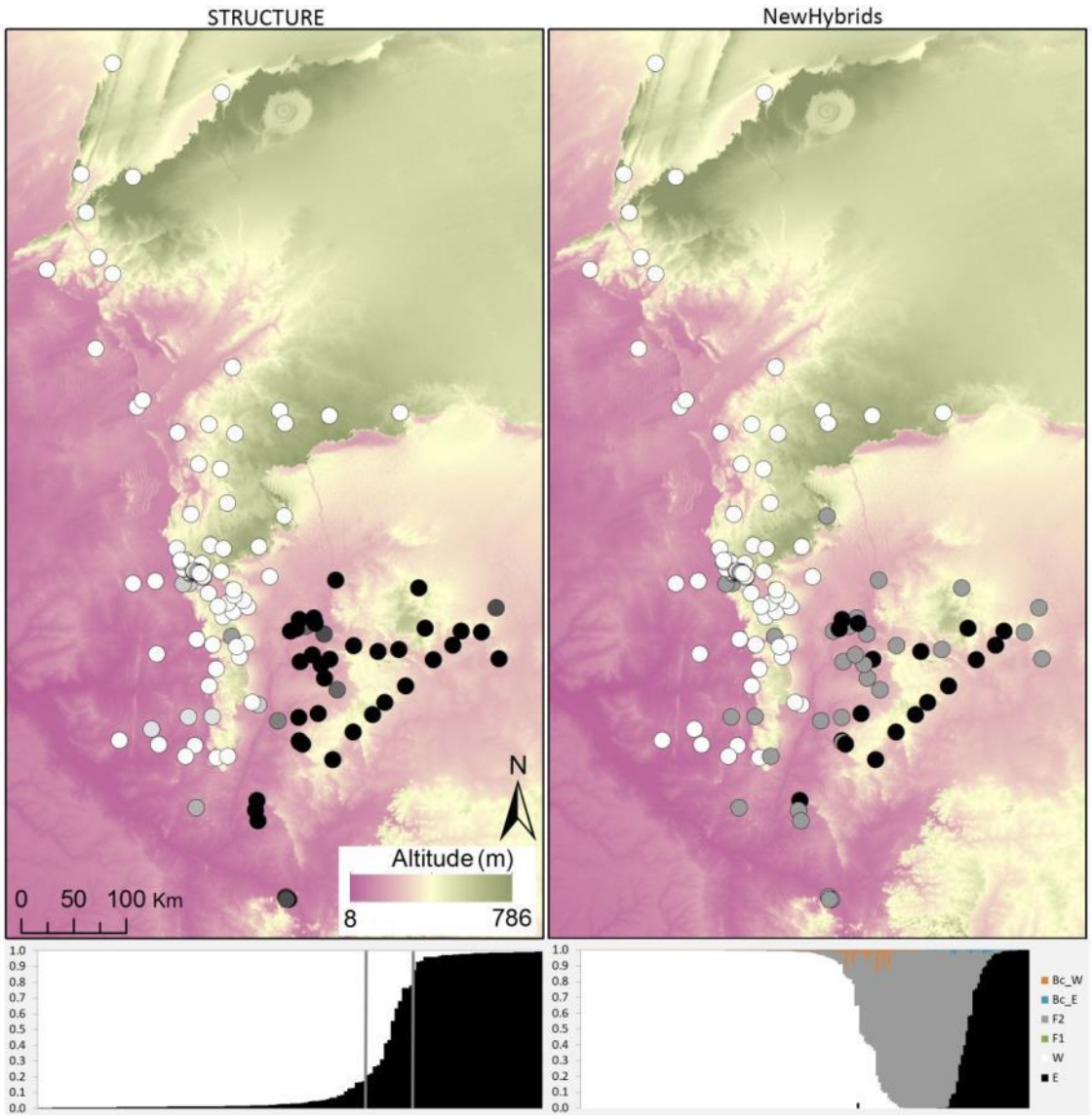

Figure 6. 2. Population structure and genetic diversity of *Agama boulengeri*. STRUCTURE (left) and NewHybrids (right) results. Samples in the structure plot were ordered by affinity to each cluster, in order to indicate individuals with less than 80% affinity to a cluster (grey vertical bars); these intermediate individuals are coloured in shades of grey (white=0, black=1) according to the affinity to cluster µ-E. In the NewHybrids map only individuals classified as hybrids (>80% affinity) are coloured in grey.

4. Discussion

This study is, to our knowledge, the first to assess the population structure of a Sahara-Sahel terrestrial vertebrate with high-resolution markers. Results showed there is gene flow among populations, revealing a mito-nuclear discordance in a species with deeply divergent lineages. Old intra-specific lineages seem to be common in reptiles from the Sahara-Sahel and other deserts (Pepper et al., 2011a; Wood et al., 2013; Brito et al., 2014), but contemporary admixture has rarely been reported. However, hybridization between old lineages at lower latitudes does seem to be more common than in

temperate regions (Hewitt, 2011). This significantly affects previous assumptions about *A. boulengeri* and the biogeographic history of the area, and stresses the importance of assessing the more recent gene flow patterns in arid landscapes in North Africa to understand the evolutionary patterns at work in the Sahara-Sahel, and conservation purposes. However, in order to reach a level of biogeographic knowledge comparable with better studied desert regions, many more species need to be studied, be it in terms of distribution, phylogeographic patterns, population structure or landscape genetics.

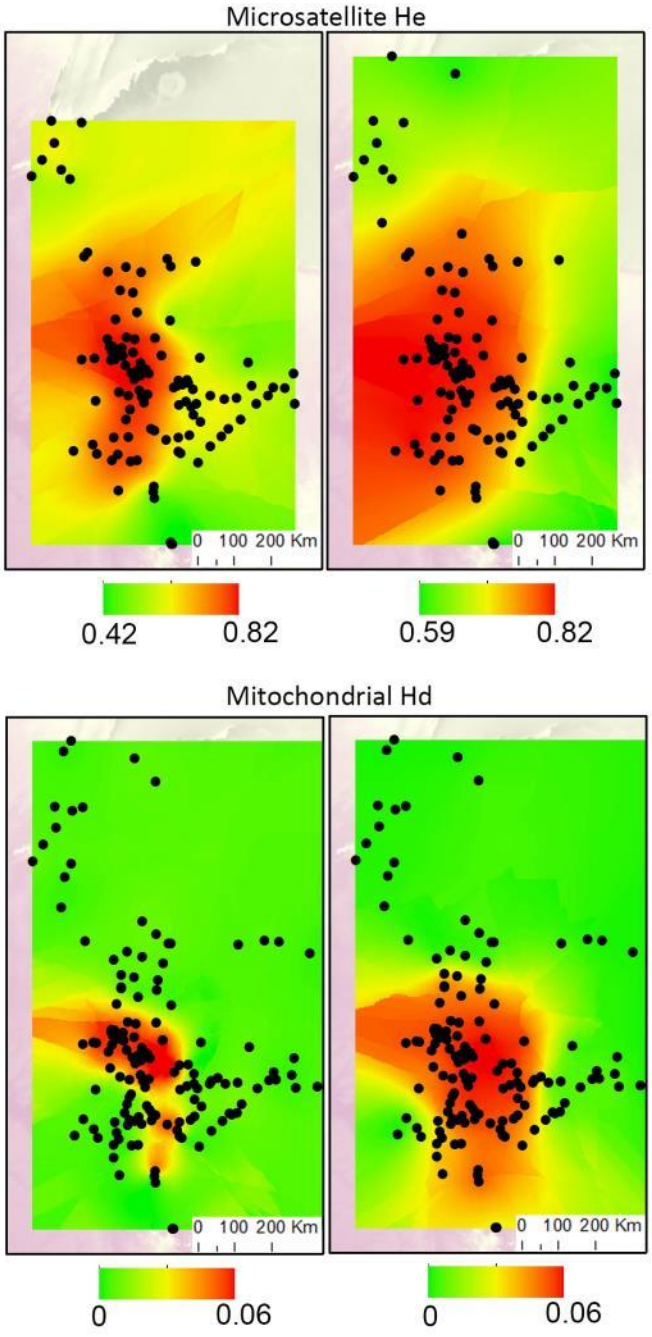

Figure 6. 3 Interpolation maps of genetic diversity, using a radius of 45 (left) and 100 (right) km; black dots represent the samples used in each interpolation. *He* = expected heterozygosity; Hd = haplotype diversity.

The empirical observation of *A. boulengeri* being highly dependent on the availability of or proximity to rocky outcrops was now confirmed by the ecological models. This indicates that previous failure in detecting this relation was due to the coarse resolution or low accuracy of previously available land-cover layers for the region (Vale et al., 2012), demonstrating the usefulness of the new land-cover layers (Campos & Brito, *in prep.*), and also supports the notion of more sandy valleys working as filters or barriers to dispersal and gene flow. However, stronger connection between landscape resistance and genetic distance were not detected, in comparison to when using Euclidean distances alone. The sampling of *A. boulengeri* here presented expands beyond the known distribution of the species thus calling for an update to the respective IUCN assessment. This work also illustrates the importance of the Djouk and Karakoro valleys, and not just the surrounding mountains, for the preservation of evolutionary and climate-change response dynamics, adding to its importance as biodiversity-rich region (Velo-Antón et al., 2014; Vale et al., 2016).

4.1. Genetic structure

The simplest explanation for the parapatric distribution of the four mitochondrial lineages of *A. boulengeri* would be vicariance, with aridification and sand encroachment isolating the populations in different mountain systems, a pattern observed in the Sahara (Metallinou et al., 2015) and other deserts (Holycross et al., 2002; Pepper et al., 2011a). However, the presence of an unsuitable area within the range of mito-lineage N (Vale et al., 2012) larger than the sandy valleys separating mito-lineages N and S (Djouk) and N+S and E (Karakoro), and the fact that lineage split times do not reflect geographic proximity (Fig. 6.1; Gonçalves et al., *in prep.*), reveal that the species' history is more complex. The pattern of nuclear gene haplotype sharing, however seems to be more related to the landscape than mitochondrial lineage divergence history, with the further sand-isolated mito-lineage E lineage being more genetically distinguishable, and mito-lineages N and S showing extensive haplotype sharing , (Fig. 6.1, Gonçalves et al, *in prep.*). Incomplete lineage sorting couldn't be ruled out as an explanation for this pattern (Gonçalves et al., *in prep.*), but the Miocene crown age of *A. boulengeri*, the low nuclear haplotype diversity in comparison with other *Agama* species, and the coherence of nuclear sequence marker spatial structure with the landscape, raised the possibility of secondary contact or hybridization among previously isolated entities. The gene-flow among mitochondrial lineages observed in the microsatellite clustering supports the later, thus excluding the hypothesis of divergent lineages with incomplete lineage sorting.

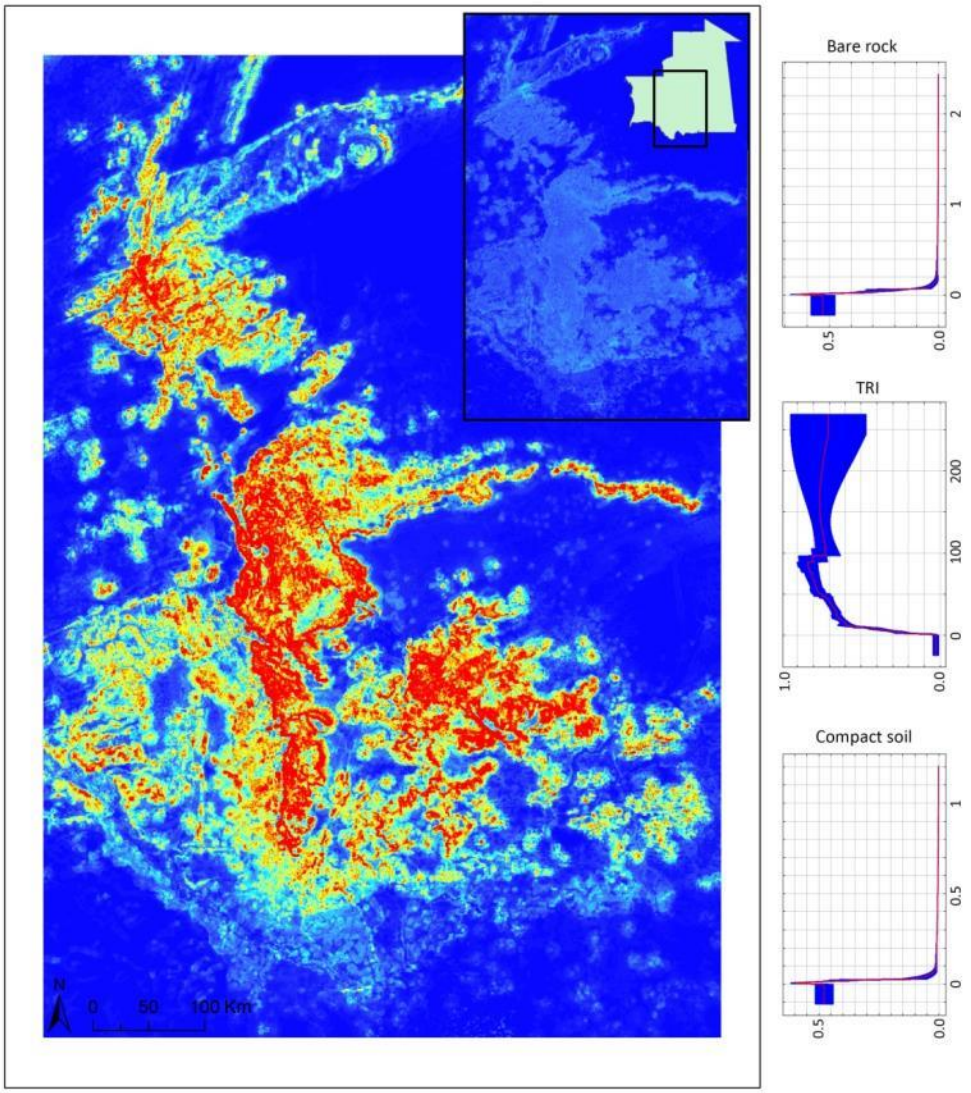

Figure 6. 4. Ecological niche-based model for *Agama boulengeri*, using topoclimatic and land-cover variables. LEFT: average prediction occurrence probability from 25 replicate models and standard deviation of predictions (small inset); red represents high probability (and SD) of occurrence (blue represents low); the range of the map is the same as in Fig. 2. RIGHT: Response curves of the variables with highest cumulative contribution (77.1%) depicting probability of occurrence along the environmental gradients. TRI = Terrain roughness index.

The East-West structuration of microsatellite diversity is also more coherent with the connectivity of rocky habitat and the occurrence models than with the mitochondrial phylogeny, explaining the nuclear gene haplotype sharing (Figs. 6.1-4). But even if admixture explains that, nuclear gene diversity is still lower than expected considering the mitochondrial one. Although mitochondrial lineages coalesce faster than nuclear ones given the different effective population sizes (Hudson & Turelli, 2003), it is highly unlikely that the observed mitochondrial and nuclear gene geographic structures (Fig. 6.1) have arisen through the same dispersal and vicariance events or even occurring in the similar temporal frame. Rather, the nuclear gene diversity seems to be much younger. The low microsatellite F_{ST} among μ-W and μ-E also indicates that the divergence between these two entities is low and possibly recent.

4.2. Mito-nuclear discordance

In cases of mito-nuclear discordance like this, with higher structuration in mtDNA, the most likely explanations are strong selection on the mitochondria (usually accompanied by introgression) or sex-biased asymmetries (Toews & Brelsford, 2012). Such asymmetries can be caused by male-biased dispersal, or sex-biased offspring production. The latter is usually due to decreased fitness of one of the sexes of the offspring, mostly the heterogametic, but given *Agama* has a temperature-dependent sex determination (Charnier, 1966; El Mouden et al., 2001), the chromosomal composition should not be the case of the observed pattern. Given the latitudinal belt-like distribution of climatic regions in the Sahara-Sahel, selection on the mitochondria would be expected to produce a latitudinal or elevation-related spatial differentiation, as it has been observed for instance in hares (Melo-Ferreira et al., 2009). However, that is not the case, with the range of each lineage being clearly delimited by sandy valleys between mountains. Since each lineage is separated from the other by a barrier (even if small and/or porous), female philopatry and male-based dispersal (MBD) would be a stronger *a priori* hypothesis. On the one hand, selection on the mitochondria could still be responsible for diversification through mechanisms like mito-nuclear compatibility (Dowling et al., 2008; Bar-Yaacov et al., 2015), but different markers are needed to assess such hypothesis. On the other hand, MBD has already been proposed for several species in the family Agamidae (e.g. Ujvari et al., 2008; Urquhart et al., 2009; Schofield et al., 2012), and it would be expectable in a species with territorial males, but in order to confirm it individual dispersal or local scale population genetic assessments would be needed.

4.3. Vicariance and allopatry

The presence of barriers in the landscape suggests a role for allopatric isolation in population diversification, but that might not be strictly necessary. In one end of the spectrum of possibilities, an allopatric differentiation of the four main lineages followed by secondary contact and nuclear introgression could produce the current diversity pattern (Toews & Brelsford, 2012), but a total displacement of the “resident” nuclear diversity would also be required. Also, in such scenario the observed mitochondrial diversity would be difficult to explain. Multiple introgression events into the invading populations would have occurred, but that should have left traces in the nuclear genes. The other end of the spectrum would be a group of populations in permanent contact, whose low nuclear diversity could be due to (probably recurrent) bottlenecks that would cause the loss of the less common and peripheral alleles (Allendorf, 1986). In either

case, the mitochondrial diversity would persist in local allopatric refugia in each mountain. A scenario closer to the second case is the most likely one, as the Pliocene-Pleistocene climatic oscillations would periodically create unsuitable conditions in the outer regions of the species' distribution. Increased hydric erosion and decreased aeolian sand deposition during more humid periods could have exposed rocky outcrops in the Djouk and Karakoro valleys and increased connectivity among populations. These putative retreats to the central range are coherent with the presence of all *A. boulengeri* mitochondrial lineages in the region of Djouk and Karakoro valleys (Gonçalves et al., *in prep.*) and the genetic diversity maps, both for microsatellites and

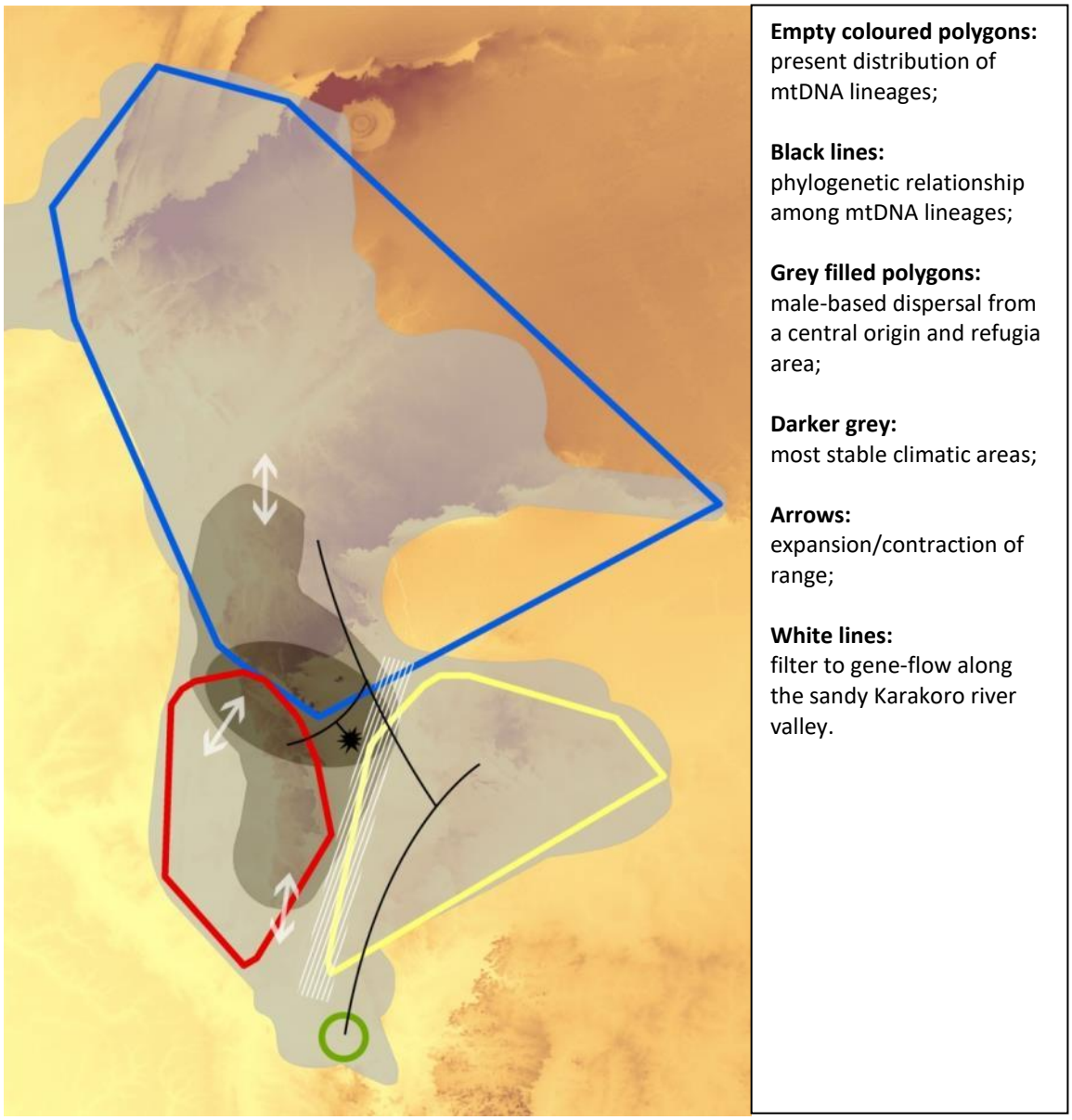

Figure 6. 5. Proposed biogeographic scenario for the evolutionary history of *Agama boulengeri*: mitochondrial diversity is maintained in *refugia* located in neighbouring mountain regions; the allopatric genetic structure is maintained by female philopatry, while male-biased dispersal from a central point-of-origin creates a pattern of higher diversity in the centre of the distribution; the lower suitability of the Karakoro River valley decreases gene-flow between East and West populations.

mitochondria, which show the highest values closer to the centre of the distribution and a decrease towards the edges (Fig. 6.3). This system would bear some similarities with a terrestrial "species pump" system, in which peripheral populations are recurrently isolated by climatic fluctuation, thus leading to speciation and evolutionary divergence (Stebbins, 1974). A putative "species pump" system involving topographic heterogeneity are the "Pleistocene Aggregate Island Complexes" (Brown & Diesmos, 2009), in which the sea acts as an intermittent barrier that drives population differentiation in spite of recurrent contacts (Papadopoulou & Knowles, 2015). In this case, a speciation event does not seem to have been complete, but such a scenario might be worth further investigation.

4.4. Desert biogeography

In light of current results, the proposed biogeographic scenario involves rock-dwelling *A. boulengeri* suffering population bottlenecks due to climatic fluctuations that led to the decrease of nuclear genetic diversity. Topographic heterogeneity allowed survival in several neighbouring refugia, which due to male-based dispersal resulted in a parapatric distribution of mitochondrial lineages (Fig. 6.5). Other processes could be involved, like mito-nuclear incompatibilities (Bar-Yaacov et al., 2015), but clarification depends on further studies. Although the species has a very strong affinity to rock (Fig. 6.4, Table 6.3), we could not demonstrate an increase in correlation when using resistance distance (least cost path or circuits) instead of Euclidean distances (Table C2). This could be due to inadequate sampling and scale. About one sample per location several kilometres apart may be enough to detect some correlation between geographic and genetic distances, it can be too coarse to allow differentiating more subtle patterns, especially considering these lizards might disperse only a few meters per generation (assuming similarity with the Caucasian rock Agamas from the closely related genus *Laudakia* – Panov & Zykova, 2016). Proper population sampling along transects across the sandy valleys and mountains is needed in order to take further conclusions.

Based on the case of *A. boulengeri*, we propose that deep genetic diversity should be found in other species with allopatric populations and no signs of divergent local adaptation between them, especially when the geographic scale is relatively small. Niche conservatism could be common among allopatric lineages or species occurring in desert mountains, possibly explaining the existence of deep lineages within a given species (Gonçalves et al, *in prep.*). Deep coalescent lineages have been found in more Saharan taxonomic groups (Brito et al., 2014; Metallinou et al., 2015), but also in other

desert regions like highlands in arid Australia (Pepper et al., 2011a,b). Both regions have suffered similar wide-ranging climatic cycles, and aridity-induced vicariance has been suggested to explain similar diversification patterns, with biotic responses to climatic fluctuation involving persistence and resilience rather than large-scale migration (Byrne, 2008; Pepper et al., 2011a). If the barriers between lineages are intermittent or porous, the secondary contacts and mito-nuclear discordance here discussed might be common as well. The higher the niche conservatism, the more likely a species is to suffer divergence through vicariance brought by the climatic fluctuations, due to inability to adapt to changing conditions (Wiens & Graham, 2005). Conversely, separate populations that keep the same niche requirements over long periods of time could have more chances to breed when they come into secondary contact, since they will likely keep similar habitat preferences, and any divergence between them is only dependent on neutral or stochastic isolation mechanisms. So although these species are more likely to diverge purely by vicariance, they could also need larger geographic separation, or much longer time to accumulate enough divergence to become different species, when compared to populations separated by vicariance events but that can more easily adapt and evolve into different niches. Given the 20-100Ka duration of the climatic cycles in North Africa, it should then be expected that other species with high niche conservatism may also show high genetic diversity, so researchers should take care not to hastily attribute substantial lineage divergence to the presence of cryptic species.

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Chapter 7

General discussion

7.1. Integrative approach

The integration of phylogenetics and eco-geographic analysis, particularly niche-based modelling approaches, has increased in recent years and allowed great advances in our understanding of the distribution and evolution of biodiversity, both at macro- and micro-biological scales. Examples include niche-informed species delimitation (e.g. Raxworthy et al., 2007), using genetic diversity to inform future distribution models (e.g. Benito Garzón et al., 2011), using future distribution models to predict genetic structure (e.g. Espíndola et al., 2012; Martínez-Freiría, 2015), studying contact zones (e.g. Tarroso et al., 2014), or understanding of evolutionary processes (e.g. Graham et al., 2004; Losos, 2008; Wiens, 2004).

Throughout this work, phylogeographic and eco-geographic tools were combined in order to study the biodiversity in the Sahara-Sahel and arid North Africa, and the evolutionary processes that have shaped the current biodiversity distribution. Phylogenetics tools were used to infer and represent evolutionary relationships, which were put into a geographic context based on the samples collection and species' distributions, sometimes informed by SDM. Population-genetics tools were mostly applied to determine population structure and detect contact zones among populations, using Bayesian model-based clustering methods (Pritchard et al., 2000); to assess demographic history, based on diversity and demographic statistics (Hamilton, 2009); or to represent reticulated evolutionary relationships, using graphs with cycles ("networks"; Bertorelle et al., 2009). Eco-geographic tools were used to visualize the inferences from the phylogeographic component, not only by representing distributions, but also by providing spatially explicit representations of evolutionary relationships or genetic diversity. Quantitative niche comparisons allowed testing hypotheses regarding the evolutionary processes behind lineage diversification. Inherently integrative

analysis like spatial interpolations of genetic diversity, or continuous phylogenetic diffusion models were able to provide insights that would be hard to acquire otherwise.

7.2. Biodiversity distribution

Increasing the knowledge about the distribution of biodiversity in North Africa was an objective addressed in almost every chapter of this thesis, and even beyond those. The effort dispended during more than 200 days of several campaigns of fieldwork resulted in substantial amounts of occurrence data, tissue samples, and ecological observations whose breadth by no means is represented in this thesis. Of course, most chapters here included incorporated data and samples collected by other researchers, but this goes to illustrate the importance of continued data collection in the field, and a contribution that goes well beyond the particular subjects here addressed. Chapter 3 comprises a direct output from this kind of endeavour, which at the time constituted a valuable contribution to the study of the herpetofauna of Termit and Tin Toumma National Nature and Cultural Reserve, the largest single protected area in Africa. Another survey has been published since then (Ineich et al., 2014), but the area remains mostly understudied. Chapters 5 and 6 also increased the available knowledge on the distributions of *Psammophis schokari*, *Hoplobatrachus occipitalis*, and the *Agama* genus. Chapter 6 is also one of the few studies in the regions to address distribution at the local scale. The present contributions, together with previous models on the species' distribution (Vale et al., 2012), likely makes *Agama boulengeri* the species of Sahara-Sahel herpetofauna with the most well-known distribution.

7.3. Tool development

Possibly having the most well-known distribution, together with occurring in a sky-archipelago in the Sahara-Sahel transition posed *Agama boulengeri* as a prime candidate for the development of fine-scale genetic markers. In the Sahara-Sahel there is an almost total absence of studies on fine-scale spatial and temporal patterns of biodiversity and evolutionary processes. There are a few, but rare, exceptions of studies employing microsatellite markers in crocodiles (Velo-Antón et al., 2014), bees (Shaibi et al., 2009; Shaibi and Moritz, 2010), plants (Migliore et al., 2013). As far as my knowledge goes, no such studies exist focusing on the evolutionary history of terrestrial vertebrates in the Sahara-Sahel. The closest examples are developed but unexplored microsatellite markers (Lopes et al., 2015), studies oriented to conservation

(e.g. Silva et al., 2014) or centred in other or larger areas but including some samples from the region (e.g. Leite et al., 2015). The optimized microsatellites in chapter 4 allow assessing the population structure and gene-flow at the fine-scale, bringing the possibility to study genetic connectivity through the landscape or biogeographic processes relevant for recent time-scales and biodiversity conservation (Bruford and Wayne, 1993; Jehle and Arntzen, 2002). Although needing optimization for any particular use, the cross-amplification of these microsatellites in other *Agama* species significantly expands the interest of these markers, given the almost pan-African distribution of the genus.

7.4. Biogeography and evolution

7.4.1. Phylogeographic patterns

Integrating a phylogenetics or population genetics component into the distribution of biodiversity has the advantage of allowing one to see beyond the present, and interpret the history of life in a given region (Avise, 2000). Throughout the manuscripts included in this work, several contributions have been made to the understanding and inferring of phylogeographic patterns. Perhaps the most pervasive was the importance of mountains and surrounding regions as refugia and centres of biodiversity. This was patent for *H. occipitalis*, with one of the lineages restricted to southern Mauritania mountains (chapter 5.3) and was particularly evident in the case of *Agama*, with all species and most intra-specific lineages exhibiting distributions associated with mountains in Morocco, Mauritania, Central Sahara, or the Red Sea coast (chapter 5.2; Gonçalves et al., 2012). The paleoclimatic and phylogenetic diffusion models derived for *A. boueti* and *A. boulengeri* also indicate that these areas were potential refugia for the survival of both species during climatic cycles, contributing towards mounting evidence of the critical importance of mountains for the conservation of local biodiversity (Brito et al., 2016, 2014 (and references therein); Vale et al., 2015; Velo-Antón et al., 2014). Of particular importance seems to be the Djouk Valley, separating the mountains of Tagant and Assaba. The climatic refugium and diversity hotspot potential of that small area was suggested by the high genetic diversity of *A. boulengeri*, both in terms of mitochondrial lineages (chapter 5.2) and microsatellite diversity (chapter 6). This is also supported by results from other studies with crocodiles (Brito et al., 2011; Velo-Antón et al., 2014) and mammals (Vale et al., 2016). In general, higher richness in mountain regions stresses the importance of opportunity of altitudinal displacement in the survival of species in face of the climatic cycles.

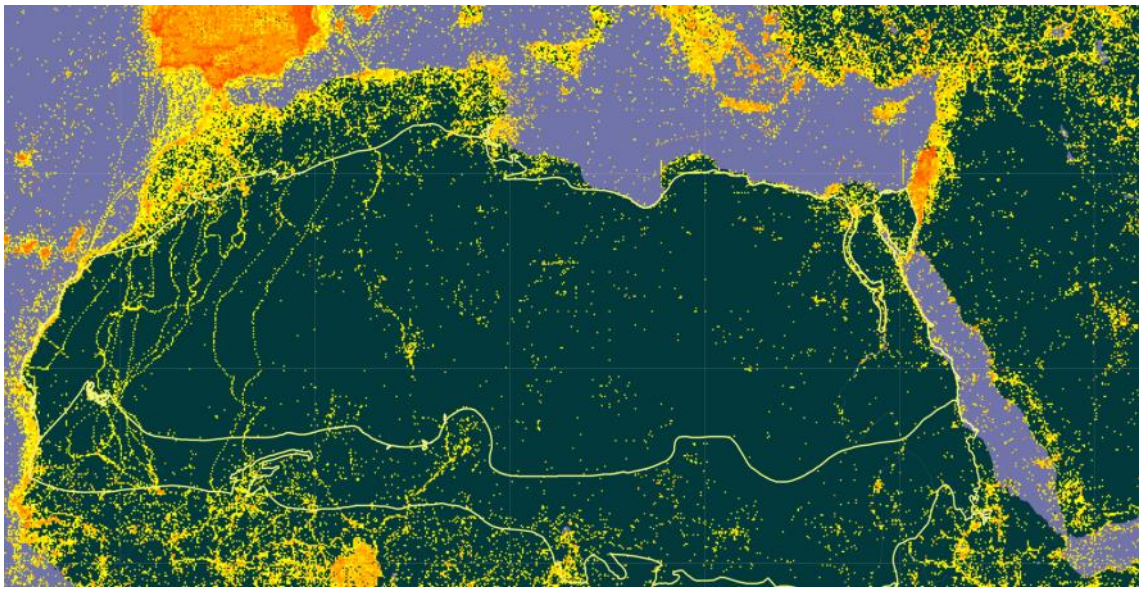

Figure 7. 1. Georeferenced presence data for all taxa stored in GBIF (accessed 01/06/2017), clearly demonstrating the considerable sampling gap in Sahara-Sahel. The yellow lines represent the approximate limits of the Sahara and the Sahel.

Paleoclimatic models allowed assessing the potential stability of the Atlantic Sahara trans-Saharan corridor for *P. schokari*, revealing not only a filter/corridor role, but also a potential refugia status for the species. Given the mesic-xeric nature of *P. schokari*, it could be expected that Atlantic Sahara also works as a permanent filter/corridor for other species with likely similar dispersal capabilities, like the Moila Snake (*Rhagerhis moilensis*) or Saw-scaled Viper (*Echis leucogaster;* Pook et al., 2008). Evidence for very recent contact between the Mediterranean coast and the mountains in southern Algeria was also found, adding to other evidence from amphibians (Nicolas et al., 2015), but a connection from there to the Sahel seems unlikely. A putative intermittent corridor was also identified in Eastern Sahara, although the low availability of precise observational data keeps this hypothesis highly uncertain.

At the local scale of southern Mauritania, the sandy Karakoro valley separating the eastern and western mountains proved to be more permeable to gene flow in a rock specialist than previously thought (chapter 6), which suggests that area might be more important to preserve evolutionary dynamics than previously assumed.

7.4.2. Evolutionary processes in the Sahara-Sahel

The history of North Africa contains several climatic and geological events that had significant impact on local biodiversity. Although it is hard to link the timing of

diversification with particular past events, by combining geo-climatic history and phylogenetic data it is possible to approximate the processes that influenced the colonization of the Sahara and the diversity of species in and around it.

In chapters 5 and 6 there are several evidences in support of climatic cyclic fluctuations, particularly aridity-induced vicariance, as a leading motor of species diversification in the region, an idea that has been also proposed by several other authors (Brito et al., 2014, and references therein). In *Psammophis schokari* (chapter 5.1), the divergence events among major lineages all took place after the end of the Miocene, mirroring the diversification patterns found in many vertebrate and reptile groups in the Late Miocene and Pliocene (Carranza et al., 2008; Geniez and Arnold, 2006; Gonçalves et al., 2012; Wagner et al., 2011), and have been associated with the onset of the Sahara desert. The roughly contemporaneous splits of North African sub-lineages after mid-Pleistocene likewise indicate persistence and subsequent isolation in Quaternary climate refugia during humid-dry cycles. Although the role of adaptation has not been assessed in this case, and indeed divergence after aridification can be attributed to ecological opportunity (e.g. Carranza et al., 2008), *P. schokari* lineage distributions were coherent with the predicted climatically stable areas, suggesting a vicariant effect of climate . In *Agama*, lineage splits do not display the contemporaneity that the *P. schokari*'s do in the Pleistocene, but a more robust analysis including phylogenies, paleoclimatic models and the niche comparisons also point in the direction of a vicariant cause of diversification. Considering the species ecology this vicariance is most likely caused by aridity. An exception seems to be *A. impalearis*, for which the barrier between intra-specific lineages seems to be cold temperatures in high altitudes. Another exception is illustrated by the Moroccan and Mauritanian lineages of *P. schokari*; allopatry here was most likely due to the Tamanrasset palaeoriver, which opened on the Atlantic coast in the north of present-day Mauritania (Skonieczny et al., 2015). No clear signs of ecological adaptation were found, but the less similar niche of the western lineage of *A. boueti*, together with indications of no allele sharing in nuclear markers could indicate the presence of cryptic species in the group.

The effects of climatic cycles were reflected not only on divergence events, but also on the distribution of species' genetic diversity. In the case of *A. boulengeri* the parts of its distribution away from the core are less diverse, particularly the northern areas. This is likely due to the fact that humid-arid cycles mostly result in latitudinal shifts of climatic zones, to which the more arid north would be more sensible. The complexity of patterns that can result from the interaction of climate and geography is also illustrated by *A. boulengeri*. In the most likely biogeographic scenario, this rock dwelling species

suffered demographic bottlenecks due to climatic fluctuations that led to the decrease of nuclear genetic diversity. Topographic heterogeneity allowed survival in several neighbouring refugia, which due to male-based dispersal resulted in a parapatric distribution of mitochondrial lineages. Even after around 6Ma of divergence among mitochondrial lineages, they seem to maintain high gene-flow (chapter 6). This case also indicates that habitat specialization might prevent range expansions under favourable conditions. Like with *A. boulengeri*, other species in the same end of the vicariance-adaptation spectrum (with high niche conservatism) could contain deep genetic diversity could be found in other species, especially when the geographic scale of occurrence is relatively small.

The frog *Hoplobatrachus occipitalis* exhibited the least structuration of genetic variability, with very little diversity when compared with the one displayed by the other study subjects for an equivalent geographic area. This signature of a strong bottleneck bottleneck followed by a considerable demographic expansion demonstrates the weight of climatic cycles. The most striking pattern, however, was the evidence for the occurrence of an allopolyploidisation event that resulted in the isolation of a diploid population in the southern mountains of Mauritania, surrounded by a tetraploid one. This shows diversification in the region can also occur with little influence from the climatic fluctuations in the Pliocene-Pleistocene.

7.5. Future prospects

Despite the contributions here presented to the understanding of the origins and distribution of biological diversity in North Africa and the Sahara-Sahel, much work is still to be done. At the most basal level is the need for further sampling, especially to cover the gaps persisting throughout the region (exemplified by data available on GBIF; Fig. 7.1). Sampling efforts oriented to unravel the evolutionary processes operating in the region are also needed, especially at the fine scale. As an example, even though the manuscript in chapter 6 is based on one of the best spatial sampling coverage available for a species in the region, it would still be considered very coarse in other regions, for instance like Europe. Assessing conservation status and delineating conservation strategies is likewise dependent on increased sampling effort.

The importance of mountain regions for conservation is becoming more and more evident, but our understanding of evolutionary dynamics taking place there is still lacking. How many species are there, how prevalent is divergence through allopatry in

these systems, how many general phylogeographic patterns are there, and how do they compare among species, are all questions that remain unanswered. This thesis focused on the biogeographic and phylogeographic patterns, and the impact of climatic cycles, in aquatic, mesic and mesic-xeric desert species. It would therefore be interesting to study xeric species on the same manner and contrast the conclusion to these results. Assessing the effect of different ecological requirements or dispersal abilities in species' evolutionary history is another open front. Comparative phylogeographic studies that address some of these questions could be the next step in Mauritania, given the already or soon-to-be available data on several taxa including mammals (Ben Faleh et al., 2012; Lado, 2015; Leite et al., 2015; Lima, 2013; Nicolas et al., 2009; Sousa, 2015), reptiles (Froufe et al., 2013; Gonçalves et al., 2012; Lopes, 2014; Melo, 2016; Velo-Antón et al., 2014) or fishes (Dilytė, 2014; Kide et al., 2016). In the same way, the amount of available data will soon allow to draw comparisons with other more studied arid regions, like North America or Australia. Still, in general, more species need to be studied in order to understand the diversity and evolutionary dynamics in the region.

Future research could also focus on understanding the determinants of landscape connectivity at the local scale and locating potential dispersal corridors (Fig. 7.2), which would also be useful to predict the impacts of climate change and design conservation measures accordingly. The same can be said for impact of man-made landscape changes, like the Great Green Wall for the Sahara and the Sahel Initiative (http://www.greatgreenwallinitiative.org/), a pan-African programme launched in 2007 by the African Union (AU) that includes the creation of a vegetation barrier along the Sahel.

Building on top of the established biogeographic bases, future research could target questions regarding organism adaptation or ecology. For instance studying differences in ecology or fitness of diploid and tetraploid populations would not only be of evolutionary interest, but help understand their distribution. Studying the ecological requirements of *Agama* species could inform more complex models, or help understand what factors determine the species' distributions at the local scale. Also other questions, like how climate change will affect the continued survival of animals with temperature-dependent sex determination (like *Agama*), can only be answered after ecological and behavioural data collection.

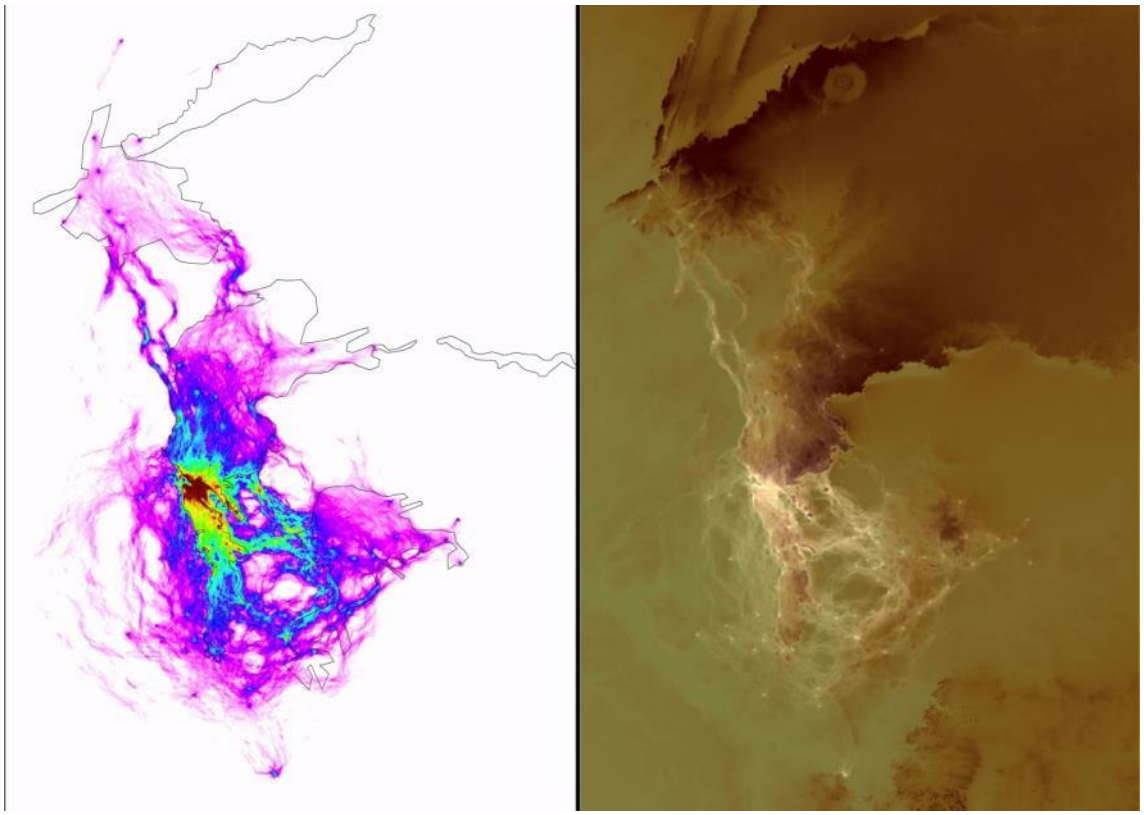

Figure 7. 2. Circuit map representing random-walk-like landscape connectivity for *A. boulengeri,* and location of putative lower resistance dispersal paths. The voltage map was calculated using Circuitscape and a resistance layer based on the map of probability of occurrence derived in chapter 6.

Future research on species distribution would benefit from the inclusion of modern techniques that could accelerate and increase genetic data gathering, such as environmental meta-barcoding for detecting the presence of multiple species from a single sample (Taberlet et al., 2012). High-throughput sequencing techniques could also be used to better understand evolutionary processes, for instance to quickly sequence multiple genetic markers in hundreds of samples for phylogenetic reconstruction (McCormack et al., 2013), or to understand the origin of tetraploidy in *Hoplobatrachus* (Session et al., 2016). The massive amount of markers obtainable from one sample would allow studying in the detail the amount and history of gene-flow among *A. boulengeri* populations.

This thesis is part of a group of ground-laying works that address the phylogeographic and biogeographic history of the Sahara-Sahel (e.g. Brito et al., 2014, and references therein; Metallinou et al., 2015; Tamar et al., 2016a, 2016b), and will hopefully be a useful stepping stone for future research on the varied fields that relate to the findings here reported, including ecology, evolution, or biodiversity conservation in the region.

7.6. Concluding remarks

By integrating phylogeographic and eco-geographic data and tools, this study has contributed to a better understanding of the patterns of biodiversity distribution and the underlying evolutionary mechanisms and diversification processes that shaped them. In summary, the main achievements and conclusions are:

1. The distribution knowledge on several species in the Sahara-Sahel has been extended, establishing a base to pursue further questions regarding their ecology and biogeography.
2. Species and lineage allopatry/parapatry was a dominant pattern in the spatial structure of genetic diversity. The evolutionary relationships among species and lineages of lizards (*Agama*), snakes (*Psammophis*) and frogs (*Hoplobatrachus occipitalis*) have been clarified, and were used as support for further analysis regarding the evolutionary processes at play in the region.
3. Climate has been shown to have played a major role in shaping the biodiversity patterns and evolution in the region, by shaping distributional ranges, causing demographic fluctuations and local extinctions, restricting dispersal, which ultimately led to diversification and speciation events. Selective pressures towards adaptation to novel conditions are expected to have played a role in diversification as well, although in the study cases here presented the signal for adaptation no strong indication of divergent adaptation was found. Although most climate-induced changes are complex, two almost binary-state features were identified as likely responsible for some of the observed patterns: the exposed rock surface, controlled by aeolian sand-deposition (shaping connectivity in *A. boulengeri*), and the Tamanrasset paleoriver (likely a barrier for *P. schokari* while functional or as a dry basin).
4. The hypothesis of vicariance as the lead motor in the diversification in *Agama* genus in North Africa was tested, and supported, by assessing the occurrence of expected biogeographic patterns and through niche comparisons in the light of Phylogenetic niche conservatism concept.
5. Non-climate related diversification mechanisms were also identified in the region, with a possible allopolyploidisation in *H. occipitalis*, which isolated in a diploid mountain-endemic population in Mauritania, surrounded by a widespread tetraploid lowland one.
6. The permanence of the Atlantic Sahara mesic corridor was supported, at least for the species tending to the xeric and high-dispersal end, like *P. schokari*. This area seems to also work as refugium during climatic cycles. The connectivity

between Mediterranean coast and southern Algerian mountains was higher than expected. Other intermittent corridors were hypothesised for eastern Sahara.

7. Evidence was provided for the importance of mountain regions as biodiversity hotspots and for their role as refugia during climatic cycles in the Plio-Pleistocene. The Djouk valley, separating the Tagant and Assaba in Mauritania, seems to be of particular interest for conservation since it contains all lineages of A. boulengeri and considerable diversity from other distinct taxa.
8. New markers have been developed in order to study *A. boulengeri* and other species in the same genus at the local populational scale, allowing to pursue questions regarding gene flow, landscape connectivity, systems of mating and dispersal, among others.
9. *Agama boulengeri* revealed a rather complex biogeographic pattern, with mitochondrial lineages diverging in the Miocene showing no signal of decreased gene flow. This rock dwelling species suffered demographic bottlenecks due to climatic fluctuations that led to the decrease of nuclear genetic diversity. Topographic heterogeneity allowed survival in several neighbouring refugia, which due to male-based dispersal resulted in a parapatric distribution of mitochondrial lineages. The results also demonstrated that apparent barriers, like the Karakoro river valley, might be important for conserving evolutionary dynamics and preserving gene-flow.
10. Based on the obtained insights, a series of questions and suggested directions for further research has been collected, which might prove useful for designing and framing future works on subjects such as phylogeography, evolutionary ecology or biodiversity conservation.

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Chapter 8

Appendices

Appendix A – Supplementary material of chapter 5.1

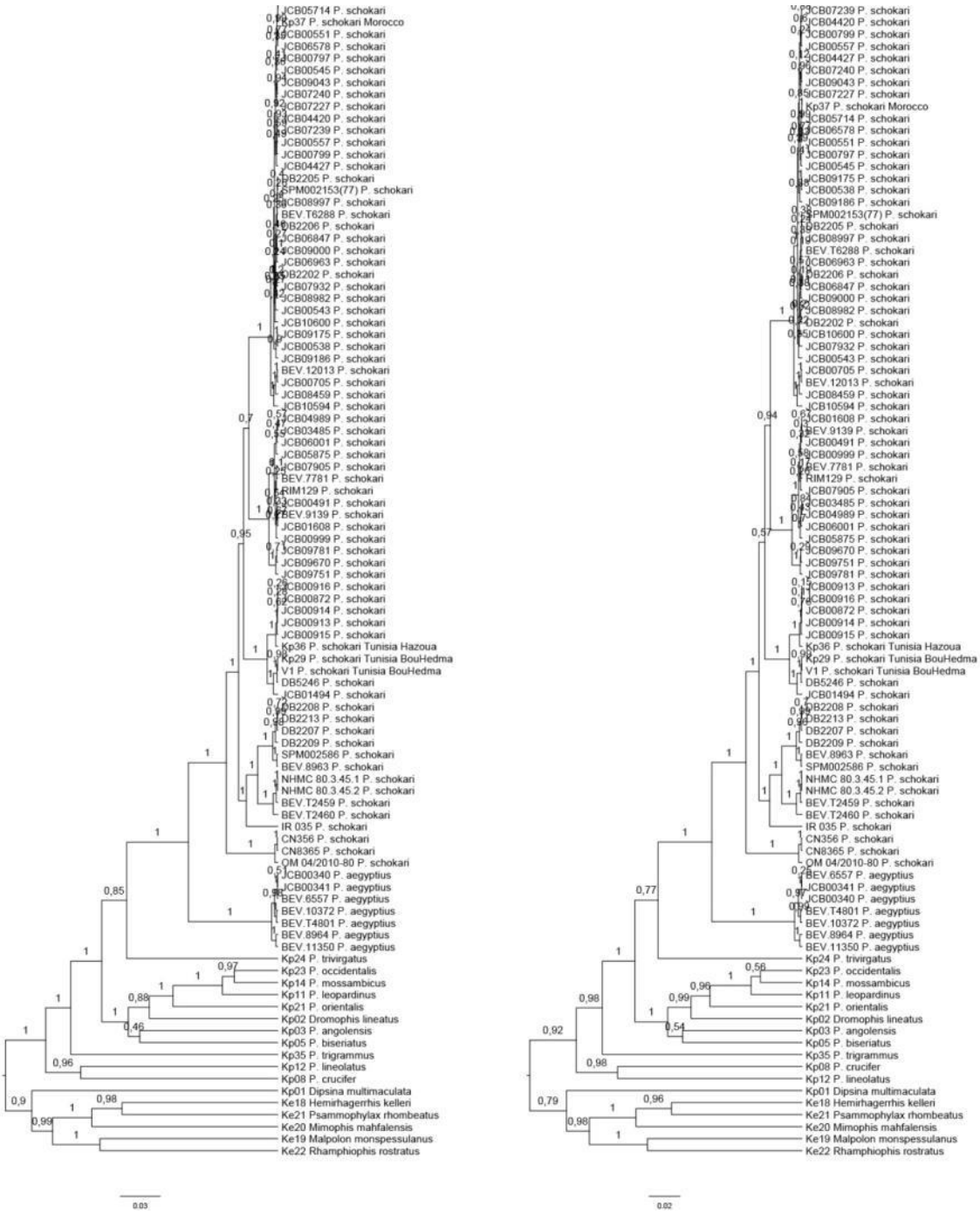

Figure A. 1. BEAST trees using codon (left) and gene (right) partitioning and the same datasets depicted in Figure 5.2.

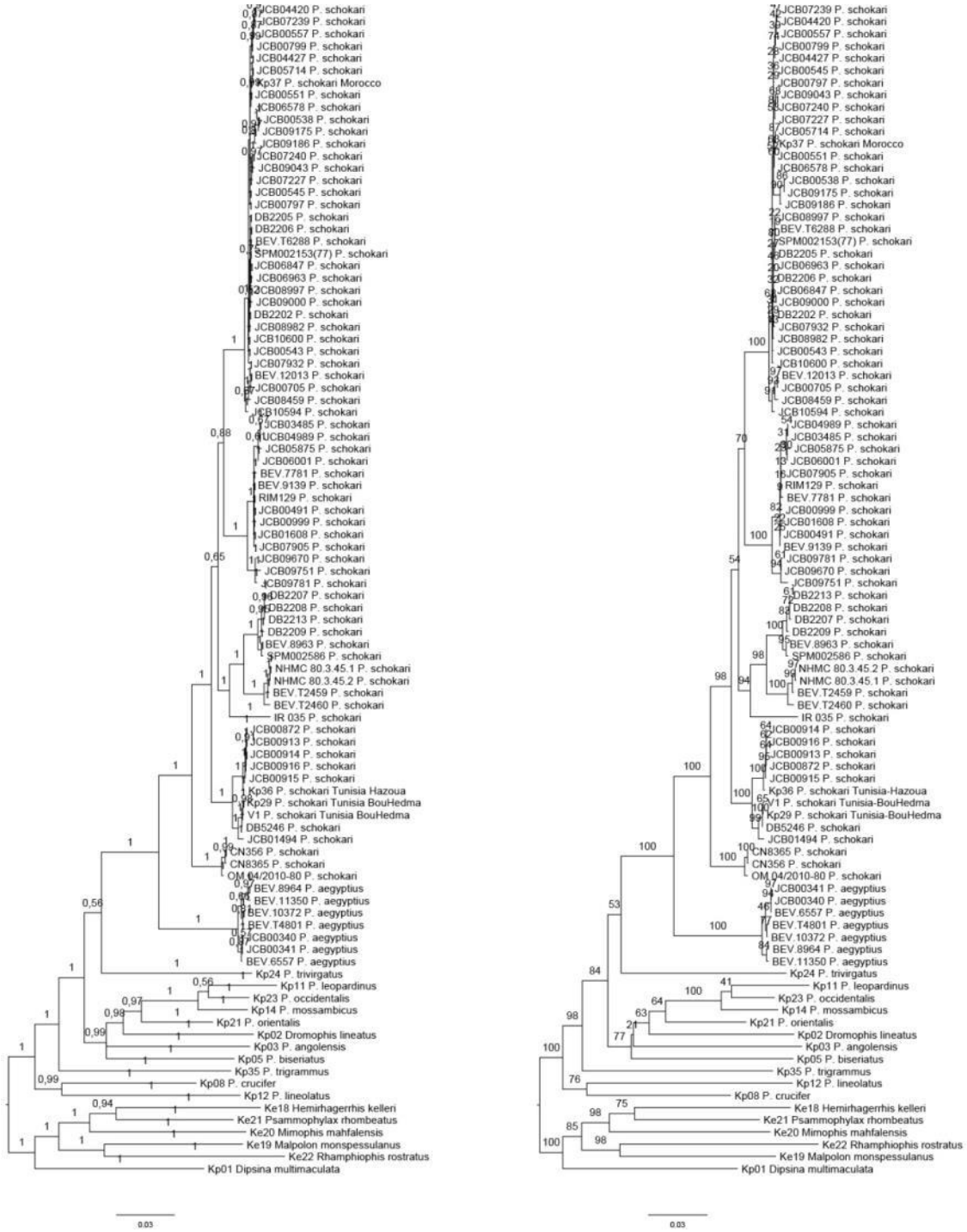

Figure A. 2. MrBayes (left) and RAxML (right) trees using gene partitioning and the same datasets depicted in Figure 5.2.

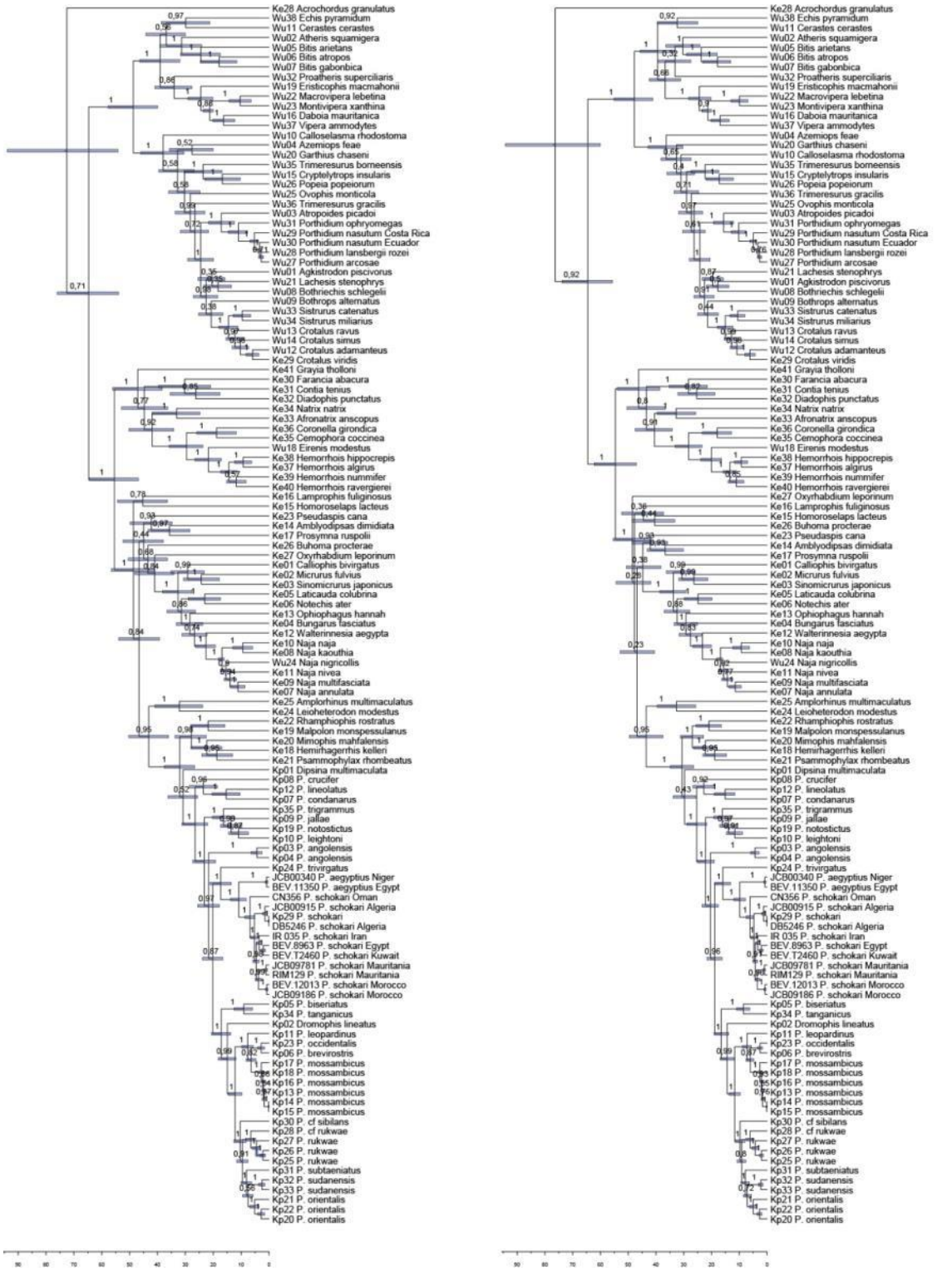

Figure A. 3. Calibrated BEAST phylogenies using gene partitioning (left) and codon partitioning with Sanders et al. (2010) calibration scheme (right).

Figure A. 4. MrBayes trees using codon (left) and gene (right) partitioning, and the same dataset depicted in Figure 5.3.

Figure A. 5. RAxML trees using codon (left) and gene (right) partitioning, and the same dataset depicted in Figure 5.3.

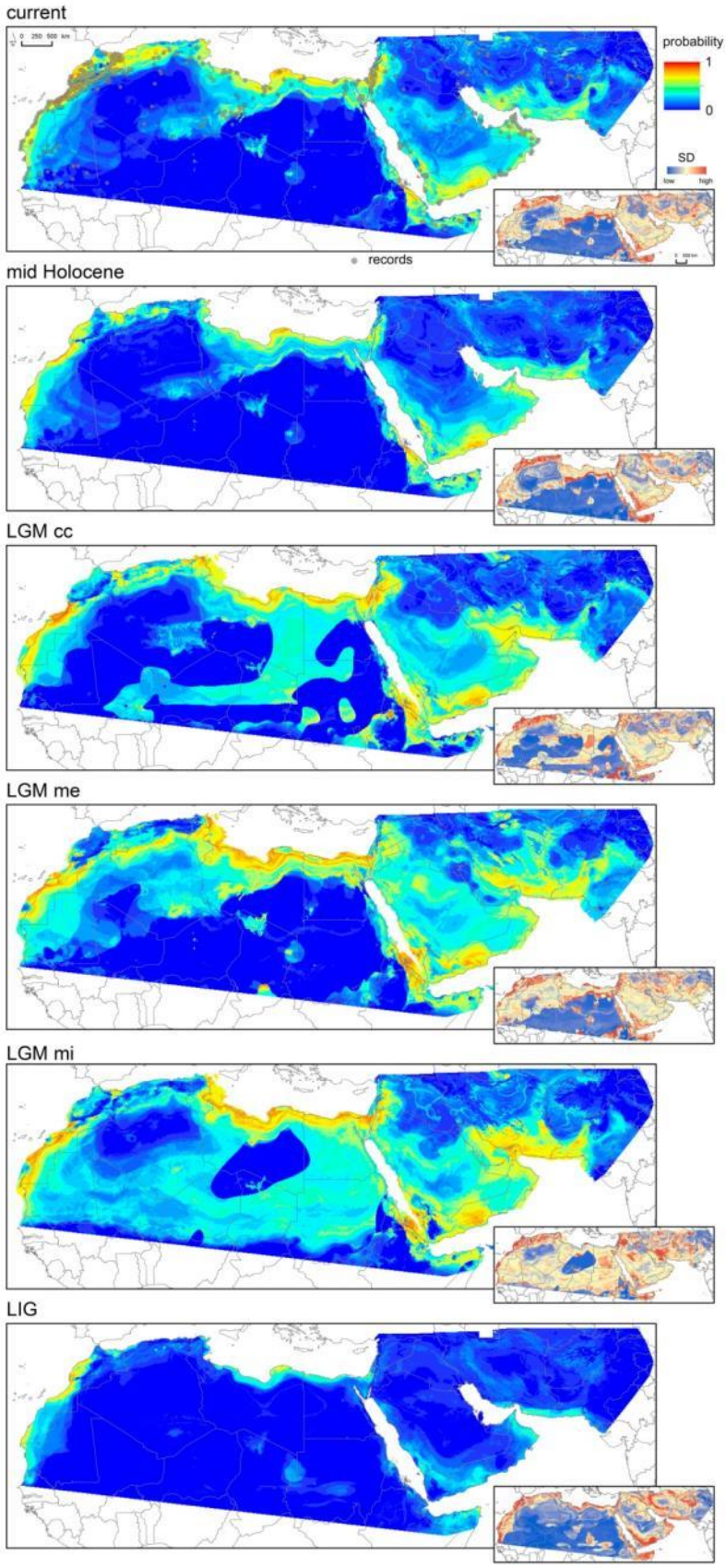

Figure A. 6. Global models: probabilistic mean models for current conditions and projections to three different past periods (mid Holocene, LGM and LIG) derived for *P. schokari* taking into account the whole species distribution at 5 arc minutes. Projections to LGM include three Global Circulation Models (see Material and Methods).

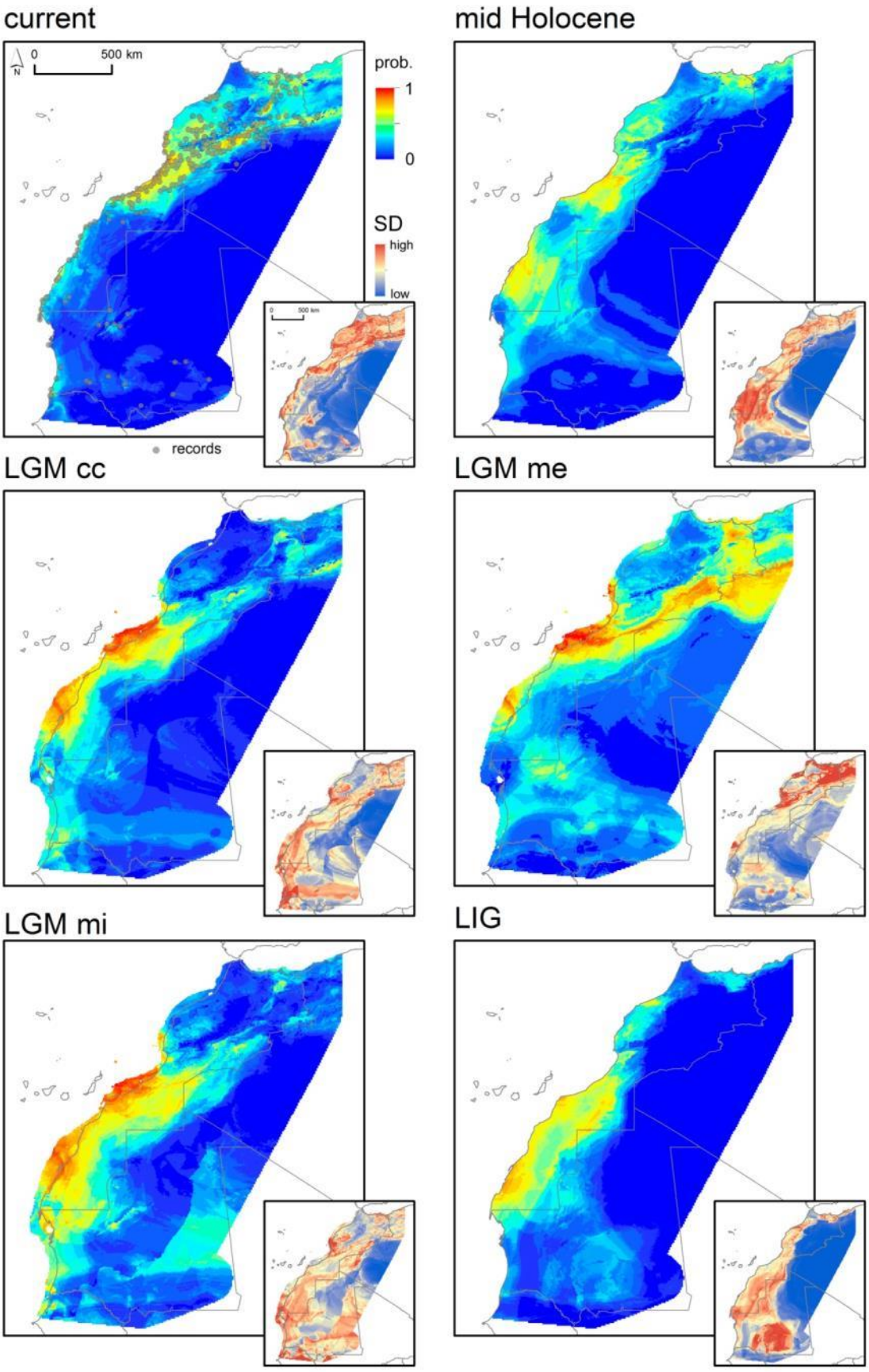

Figure A. 7. Regional models: probabilistic mean models for current conditions and projections for three different past periods (mid Holocene, LGM and LIG) derived for the West African lineages of *P. schokari* at 30 arc seconds. Projections to LGM include three Global Circulation Models (see Material and Methods).

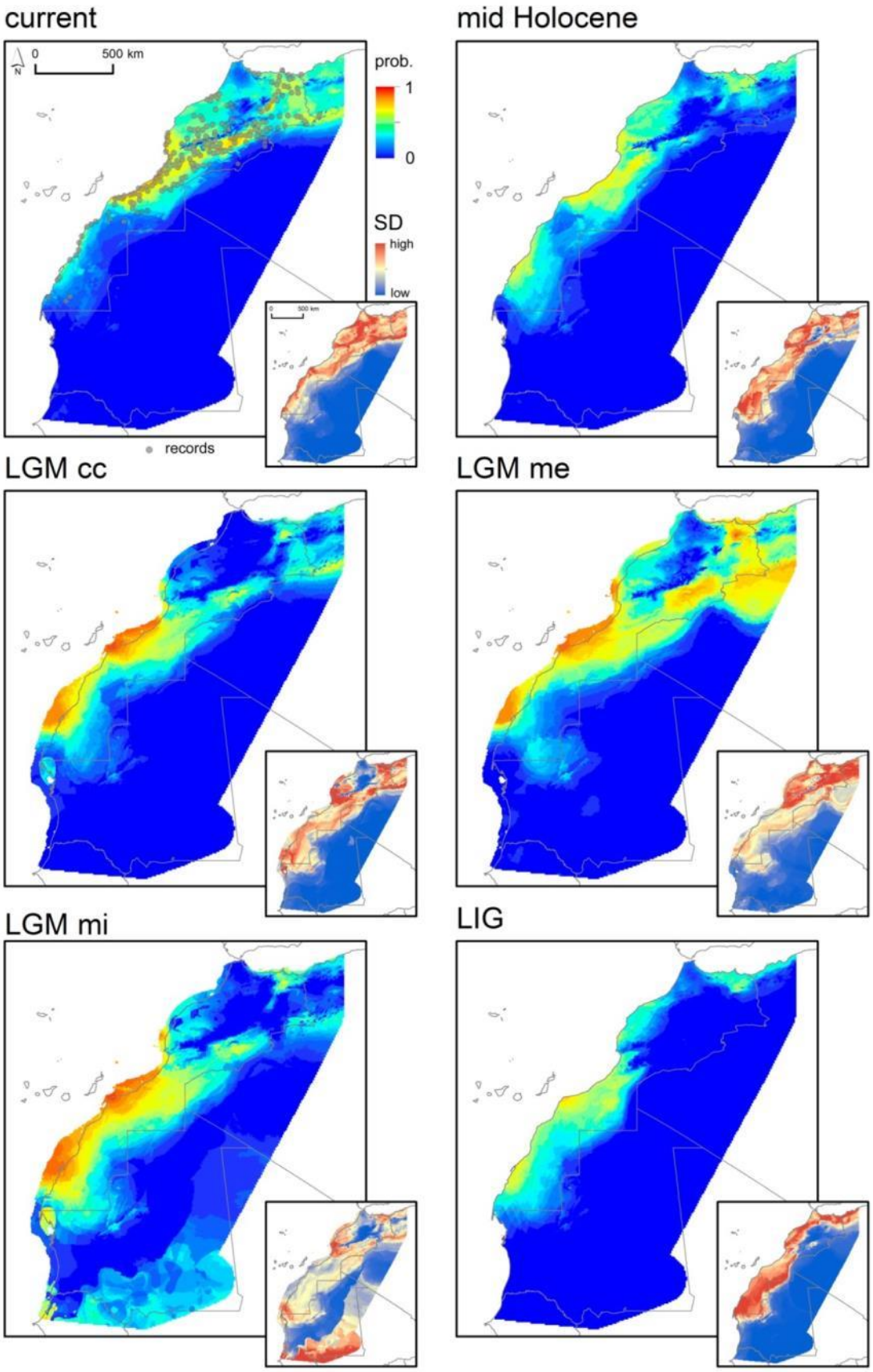

Figure A. 8. Moroccan models: probabilistic mean models for current conditions and projections for three different past periods (mid Holocene, LGM and LIG) derived for the Moroccan lineage of *P. schokari* at 30 arc seconds. Projections to LGM include three Global Circulation Models (see Material and Methods).

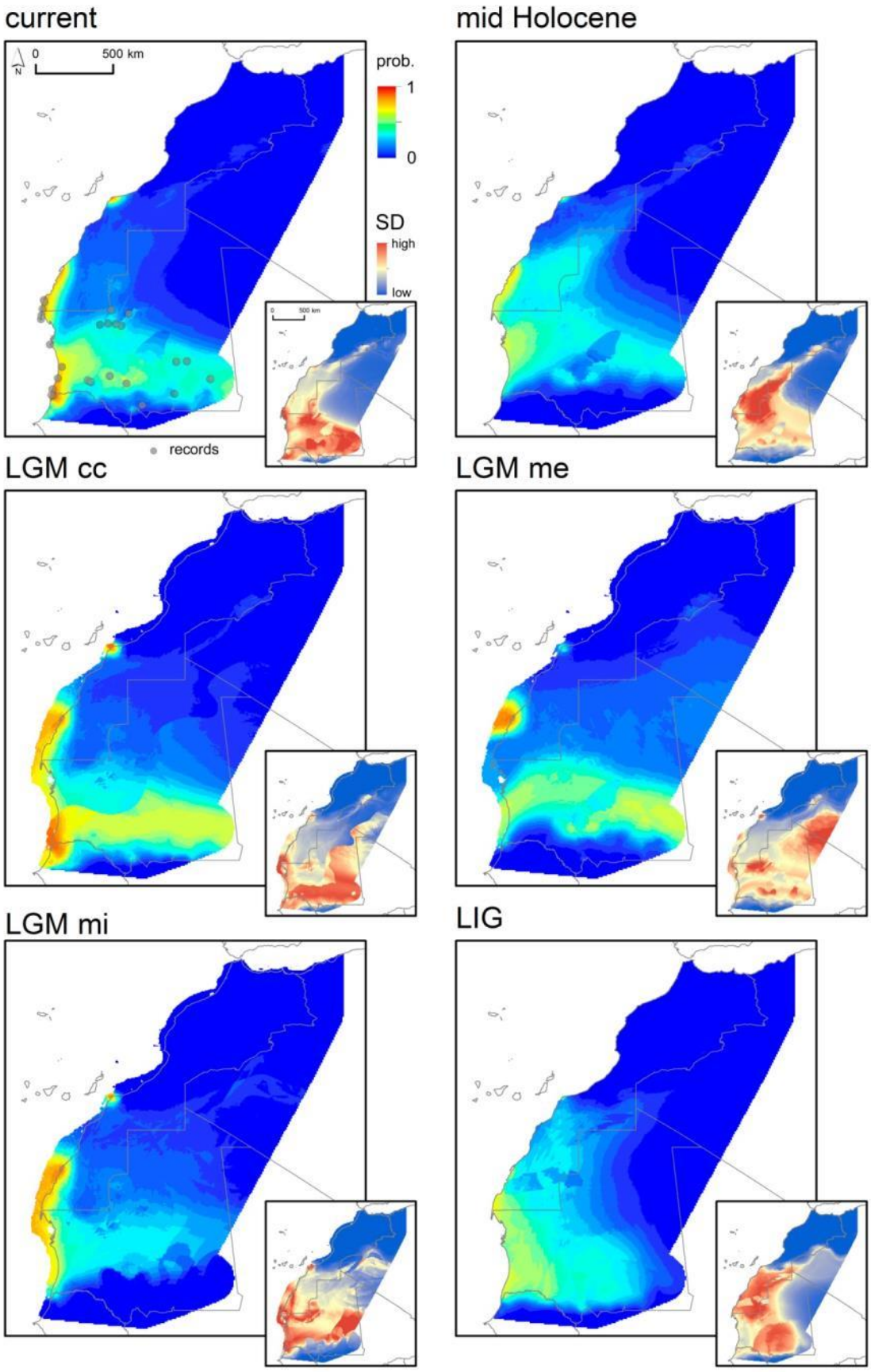

Figure A. 9. Mauritanian models: probabilistic mean models for current conditions and projections for three different past periods (mid Holocene, LGM and LIG) derived for the Mauritanian lineage of *P. schokari* at 30 arc seconds. Projections to LGM include three Global Circulation Models (see Material and Methods).

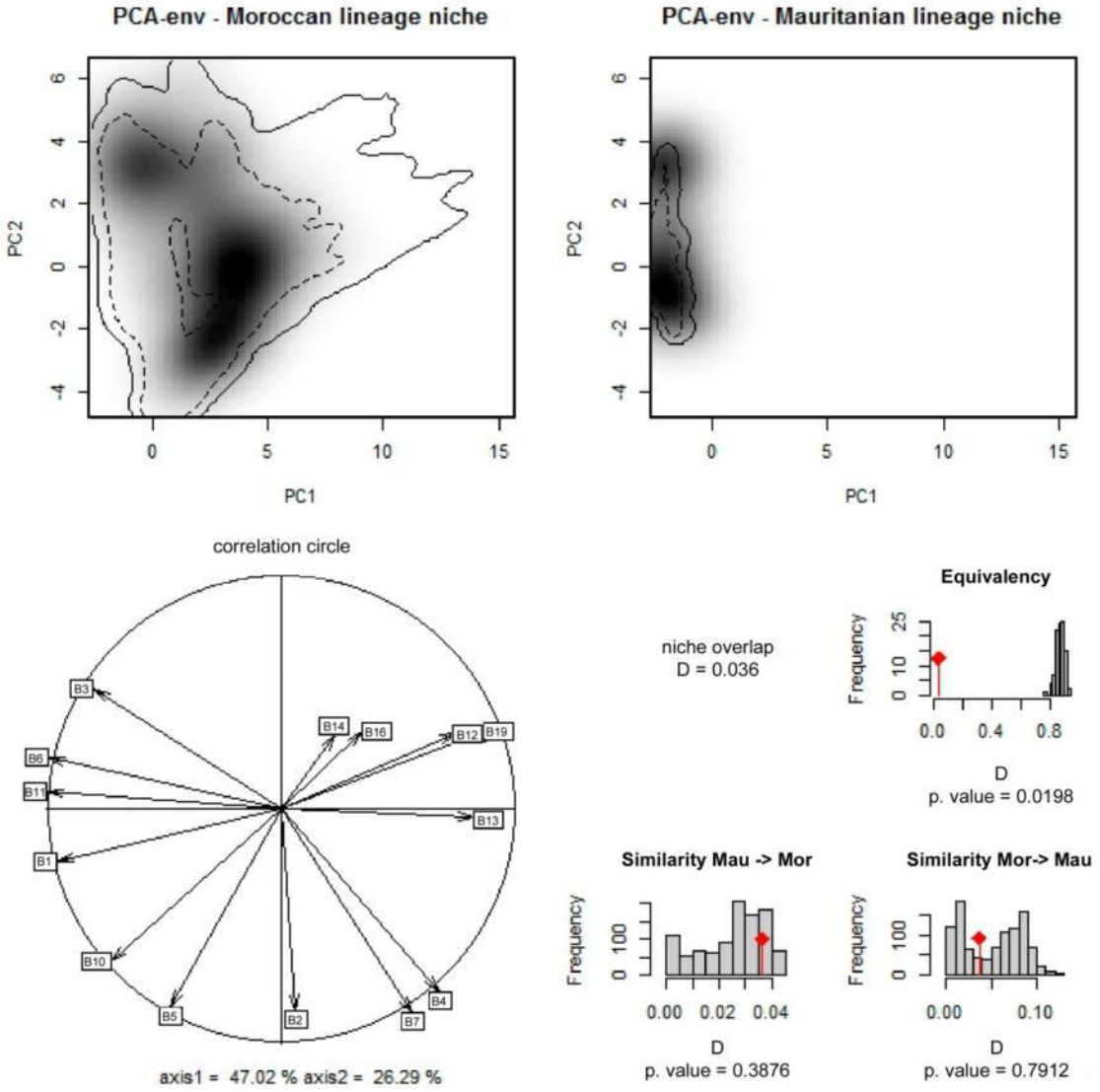

Figure A. 10. PCA-env results. Climatic niche and overlap comparisons for Moroccan and Mauritanian lineages of *Psammophis schokari*, based on calibrated Principal Component Analyses (PCA) of occurrences and climatic ranges. The top panels represent the niche of each lineage along the two first axes of the PCA in each range. Grey shading shows the density of the occurrences of the species by cell. The solid and dashed contour lines illustrate, respectively, 100% and 50% of the available (background) environment. The bottom left panel (correlation circle) shows the contribution of the climatic variables on the two axes of the PCA and the percentage of inertia explained by the two axes. In the bottom right are depicted the observed niche overlap (Schoener's *D* metric), and histograms showing niche equivalency and similarity tests with associated probability. The niche occupied by both lineages is different, but this pattern could not be dissociated from the differences in availability of local climatic conditions.

Table A. 1. Samples used for the genetic analyses, including sequences obtained from GenBank and previous publications. Coordinates are provided for the *P. schokari* and *P. aegyptius* samples used in the phylogenetic analysis represented in Figure 5.2. Samples in Dataset 1 are marked with [#], and those in both datasets with [*#].

Sample	Source	Species	Country	Latitude	Longitude	CYTB	ND4	c-mos	RAG2
[#]BEV.10372	BEV	*P. aegyptius*	Egypt	24.3648	35.286	MG002975	MG003041	MG002910	MG003096
[*#]BEV.11350	BEV	*P. aegyptius*	Egypt	22.3543	31.6092	MG002972	MG003038	MG002907	MG003093
[*#]BEV.12013	BEV	*P. schokari*	Morocco	32.4862	-5.9422	MG002979	MG003045	MG002914	MG003100
[#]BEV.6557	BEV	*P. aegyptius*	Niger	20.535	8.994	–	MG003036	MG002905	–
[#]BEV.7781	BEV	*P. schokari*	Mauritania	21.47	-11.34	MG002970	MG003035	MG002904	MG003091
[*#]BEV.8963	BEV	*P. schokari*	Egypt	30.905	29.424	MG002971	MG003037	MG002906	MG003092
[#]BEV.8964	BEV	*P. aegyptius*	Egypt	22.747	35.785	MG002969	MG003034	MG002903	MG003090
[#]BEV.9139	BEV	*P. schokari*	Mauritania	21.0684	-17.0182	MG002977	MG003043	MG002912	MG003098
[#]BEV.T2459	BEV	*P. schokari*	Kuwait	28.5504	48.0185	MG002978	MG003044	MG002913	MG003099
[*#]BEV.T2460	BEV	*P. schokari*	Kuwait	28.5822	48.066	MG002973	MG003039	MG002908	MG003094
[#]BEV.T4801	BEV	*P. aegyptius*	Egypt	25.036	33.7658	MG002976	MG003042	MG002911	MG003097
[#]BEV.T6288	BEV	*P. schokari*	Algeria	32.7762	-0.8364	MG002974	MG003040	MG002909	MG003095
[#]HDB2202	HDB	*P. schokari*	Morocco	31.1167	-6.4000	MG002959	EF128005	MG002894	MG003081
[#]HDB2205	HDB	*P. schokari*	Morocco	31.1167	-6.4000	MG002960	EF128006	MG002895	MG003082
[#]HDB2206	HDB	*P. schokari*	Morocco	34.6000	-3.3333	–	EF128007	MG002896	MG003083
[#]HDB2207	HDB	*P. schokari*	Israel	31.8833	34.7000	MG002961	EF128022	MG002897	–
[#]HDB2208	HDB	*P. schokari*	Israel	31.8833	34.7000	MG002962	EF128023	–	–
[#]HDB2209	HDB	*P. schokari*	Israel	31.7667	34.6333	–	EF128024	–	–
[#]HDB2213	HDB	*P. schokari*	Israel	29.8833	35.05	MG002963	MG003029	MG002898	MG003084
[*#]HDB5246	HDB	*P. schokari*	Tunisia	34.30195	8.36072	MG002964	MG003030	MG002899	MG003085
[*#]JCB00340	JCB	*P. aegyptius*	Niger	18.9762	12.8959	MG002987	EF128025	MG002921	MG003107
[#]JCB00341	JCB	*P. aegyptius*	Niger	18.6906	12.9224	MG002988	EF128026	MG002922	MG003108
[#]JCB00491	JCB	*P. schokari*	Mauritania	17.3927	-16.0621	–	EF128008	MG002923	MG003109
[#]JCB00538	JCB	*P. schokari*	Morocco	22.0782	-16.6884	MG002989	EF128009	–	–
[#]JCB00543	JCB	*P. schokari*	Morocco	23.1798	-16.1143	MG002990	EF128010	MG002924	MG003110
[#]JCB00545	JCB	*P. schokari*	Morocco	23.1263	-16.0680	MG002991	EF128011	MG002925	MG003111

#JCB00551	JCB	*P. schokari*	Morocco	25.0440	-14.8058	MG002992	EF128012	MG002926	MG003112
#JCB00557	JCB	*P. schokari*	Morocco	26.4347	-13.9948	MG002993	EF128013	MG002927	MG003113
#JCB00705	JCB	*P. schokari*	Morocco	31.3670	-7.3922	MG002994	EF128014	MG002928	MG003114
#JCB00797	JCB	*P. schokari*	Morocco	24.7514	-14.8683	MG002995	EF128015	MG002929	MG003115
#JCB00799	JCB	*P. schokari*	Morocco	25.2074	-14.8235	MG002996	EF128016	MG002930	MG003116
#JCB00872	JCB	*P. schokari*	Algeria	32.4833	3.6500	MG002997	EF128017	MG002931	MG003117
#JCB00913	JCB	*P. schokari*	Algeria	34.8853	5.9057	MG002998	EF128018	MG002932	MG003118
#JCB00914	JCB	*P. schokari*	Algeria	34.8853	5.9057	MG002999	EF128019	MG002933	MG003119
*#JCB00915	JCB	*P. schokari*	Algeria	34.9975	6.0335	MG003000	EF128020	MG002934	MG003120
#JCB00916	JCB	*P. schokari*	Algeria	35.0480	6.1250	MG003001	EF128021	MG002935	MG003121
#JCB00999	JCB	*P. schokari*	Mauritania	17.0940	-12.0645	MG003002	MG003053	MG002936	MG003122
#JCB01494	JCB	*P. schokari*	Tunisia	22.8224	5.6028	MG003003	MG003054	MG002937	MG003123
#JCB01608	JCB	*P. schokari*	Mauritania	21.9563	-16.8751	MG003004	MG003055	MG002938	MG003124
#JCB03485	JCB	*P. schokari*	Mauritania	17.2244	-14.2150	MG003005	MG003056	MG002939	MG003125
#JCB04420	JCB	*P. schokari*	Morocco	28.8276	-10.2701	MG003006	MG003057	MG002940	MG003126
#JCB04427	JCB	*P. schokari*	Morocco	27.9493	-12.8793	MG003007	MG003058	MG002941	MG003127
#JCB04989	JCB	*P. schokari*	Mauritania	17.2668	-14.3018	MG003008	MG003059	MG002942	–
#JCB05714	JCB	*P. schokari*	Morocco	28.4501	-11.0427	MG003009	MG003060	MG002943	MG003128
#JCB05875	JCB	*P. schokari*	Mauritania	20.4543	-12.3617	MG003010	MG003061	MG002944	MG003129
#JCB06001	JCB	*P. schokari*	Mauritania	18.0596	-15.8288	MG003011	MG003062	MG002945	–
#JCB06578	JCB	*P. schokari*	Morocco	25.0479	-14.8046	MG003012	MG003063	MG002946	MG003130
#JCB06847	JCB	*P. schokari*	Morocco	28.6885	-9.3081	MG003013	MG003064	MG002947	MG003131
#JCB06963	JCB	*P. schokari*	Morocco	32.9121	-4.2262	–	MG003065	MG002948	MG003132
#JCB07227	JCB	*P. schokari*	Morocco	27.1567	-10.8472	MG003014	MG003066	MG002949	MG003133
#JCB07239	JCB	*P. schokari*	Morocco	27.0891	-11.1611	MG003015	MG003067	MG002950	MG003134
#JCB07240	JCB	*P. schokari*	Morocco	27.0537	-11.4363	MG003016	MG003068	MG002951	MG003135
#JCB07905	JCB	*P. schokari*	Mauritania	17.2768	-14.3126	MG003017	MG003069	MG002952	MG003136
#JCB07932	JCB	*P. schokari*	Morocco	30.7378	-6.1235	MG003018	MG003070	MG002953	–
#JCB08459	JCB	*P. schokari*	Morocco	31.6810	-9.6588	MG003019	MG003071	–	–

#JCB08982	JCB	*P. schokari*	Morocco	31.9803	-3.2617	MG003020	MG003072	–	MG003137
#JCB08997	JCB	*P. schokari*	Morocco	32.2009	-1.6236	MG003021	MG003073	–	MG003138
#JCB09000	JCB	*P. schokari*	Morocco	29.7855	-7.5873	MG003022	MG003074	–	MG003139
#JCB09043	JCB	*P. schokari*	Morocco	24.2967	-15.3336	MG003023	MG003075	–	MG003140
#JCB09175	JCB	*P. schokari*	Morocco	22.1625	-15.3379	MG003024	MG003076	MG002954	MG003141
*#JCB09186	JCB	*P. schokari*	Morocco	21.8846	-15.5730	MG003025	MG003077	MG002955	MG003142
#JCB09670	JCB	*P. schokari*	Mauritania	17.3499	-7.1797	MG003026	MG003078	MG002956	MG003143
#JCB09751	JCB	*P. schokari*	Mauritania	18.3905	-8.5543	MG003027	MG003079	MG002957	MG003144
*#JCB09781	JCB	*P. schokari*	Mauritania	18.3477	-9.1749	MG003028	MG003080	MG002958	MG003145
#JCB10594	JCB	*P. schokari*	Morocco	29.6864	-9.0770	MG002965	MG003031	MG002900	MG003086
#JCB10600	JCB	*P. schokari*	Morocco	29.6094	-10.0206	MG002966	MG003032	MG002901	MG003087
#NHMC_80.3.45.1	NHMC	*P. schokari*	Syria	34.3619	38.174	MG002967	–	–	MG003088
#NHMC_80.3.45.2	NHMC	*P. schokari*	Syria	34.3619	38.174	MG002968	MG003033	MG002902	MG003089
*#CN356	SC.IBE	*P. schokari*	Oman	23.1604	57.4233	MG002980	MG003046	MG002915	MG003101
#CN8365	SC.IBE	*P. schokari*	Oman	25.8640	56.2672	MG002981	MG003047	MG002916	MG003102
*#IR 035	SC.IBE	*P. schokari*	Iran	35.1109	50.8977	MG002982	MG003048	–	–
#OM 04/2010-80	SC.IBE	*P. schokari*	Oman	21.9533	59.6112	MG002983	MG003049	MG002917	MG003103
*#RIM129	SC.IBE	*P. schokari*	Mauritania	21.3776	-12.9764	MG002984	MG003050	MG002918	MG003104
#SPM002153(77)	SC.IBE	*P. schokari*	Algeria	34.3717	2.6794	MG002985	MG003051	MG002919	MG003105
#SPM002586	SC.IBE	*P. schokari*	Egypt	30.73	33.33	MG002986	MG003052	MG002920	MG003106
*#Kp01	Kelly et al., 2008	*Dipsina multimaculata*				DQ486357	DQ486332	DQ486181	–
*#Kp02	Kelly et al., 2008	*Dromophis lineatus*				DQ486426	DQ486263	–	FJ404426
*#Kp03	Kelly et al., 2008	*P. angolensis*				DQ486410	DQ486248	DQ486189	–
Kp04	Kelly et al., 2008	*P. angolensis*				DQ486416	DQ486254	–	–
*#Kp05	Kelly et al., 2008	*P. biseriatus*				DQ486389	DQ486228	–	–
Kp06	Kelly et al., 2008	*P. brevirostris*				DQ486395	DQ486234	–	–
Kp07	Kelly et al., 2008	*P. condanarus*				AF471075	AY058987	AF471104	–
*#Kp08	Kelly et al., 2008	*P. crucifer*				DQ486397	DQ486236	DQ486188	–
Kp09	Kelly et al., 2008	*P. jallae*				DQ486409	DQ486247	–	–

Kp10	Kelly et al., 2008	*P. leightoni*				DQ486467	DQ486303	DQ486197	–
*# Kp11	Kelly et al., 2008	*P. leopardinus*				DQ486456	DQ486292	–	–
*# Kp12	Kelly et al., 2008	*P. lineolatus*				DQ486450	DQ486286	DQ486195	FJ404392
Kp13	Kelly et al., 2008	*P. mossambicus*				DQ486373	DQ486212	–	–
Kp14	Kelly et al., 2008	*P. mossambicus*				DQ486383	DQ486222	DQ486185	FJ404393
Kp15	Kelly et al., 2008	*P. mossambicus*				DQ486385	DQ486224	–	–
Kp16	Kelly et al., 2008	*P. mossambicus*				DQ486392	DQ486231	–	–
Kp17	Kelly et al., 2008	*P. mossambicus*				DQ486400	DQ486239	–	–
Kp18	Kelly et al., 2008	*P. mossambicus*				DQ486411	DQ486249	–	–
Kp19	Kelly et al., 2008	*P. notostictus*				DQ486366	DQ486205	DQ486182	–
Kp20	Kelly et al., 2008	*P. orientalis*				DQ486386	DQ486225	–	–
*# Kp21	Kelly et al., 2008	*P. orientalis*				DQ486393	DQ486232	–	FJ404394
Kp22	Kelly et al., 2008	*P. orientalis*				DQ486396	DQ486235	–	–
*# Kp23	Kelly et al., 2008	*P. occidentalis*				DQ486371	DQ486210	–	–
*# Kp24	Kelly et al., 2008	*P. trivirgatus*				DQ486387	DQ486226	DQ486186	–
Kp25	Kelly et al., 2008	*P. rukwae*				DQ486375	DQ486214	–	–
Kp26	Kelly et al., 2008	*P. rukwae*				DQ486443	DQ486279	–	–
Kp27	Kelly et al., 2008	*P. rukwae*				DQ486446	DQ486282	–	–
Kp28	Kelly et al., 2008	*P. cf. rukwae*				DQ486452	DQ486288	–	–
*# Kp29	Kelly et al., 2008	*P. schokari*	Tunisia	34.5	9.5	DQ486441	DQ486277	DQ486194	–
Kp30	Kelly et al., 2008	*P. cf. sibilans*				DQ486420	DQ486257	–	–
Kp31	Kelly et al., 2008	*P. subtaeniatus*				DQ486415	DQ486253	–	–
Kp32	Kelly et al., 2008	*P. sudanensis*				DQ486382	DQ486221	DQ486184	–
Kp33	Kelly et al., 2008	*P. sudanensis*				DQ486431	DQ486268	–	–
Kp34	Kelly et al., 2008	*P. tanganicus*				DQ486378	DQ486217	DQ486183	–
*# Kp35	Kelly et al., 2008	*P. trigrammus*				DQ486458	DQ486294	DQ486196	–
# Kp36	Kelly et al., 2008	*P. schokari*	Tunisia	33.74	7.6	DQ486364	DQ486203	–	–
# Kp37	Kelly et al., 2008	*P. schokari*	Morocco	–	–	DQ486365	DQ486204	–	–
Ke01	Kelly et al., 2009	*Calliophis bivirgatus*				AF217812	AY058979	AY058934	–

Ke02	Kelly et al., 2009	*Micrurus fulvius*	AF217839	AY058980	AY058935	–
Ke03	Kelly et al., 2009	*Sinomicrurus japonicus*	AF217831	AY058971	AY058926	–
Ke04	Kelly et al., 2009	*Bungarus fasciatus*	AF217830	U49297	AY058924	–
Ke05	Kelly et al., 2009	*Laticauda colubrina*	AF217834	AY058977	AY058932	–
Ke06	Kelly et al., 2009	*Notechis ater*	AF217836	AY058981	AY058937	–
Ke07	Kelly et al., 2009	*Naja annulata*	AF217829	AY058970	AY058925	–
Ke08	Kelly et al., 2009	*Naja kaouthia*	AF217835	AY058982	AY058938	–
Ke09	Kelly et al., 2009	*Naja multifasciata*	AF217837	AY058985	AY058941	–
Ke10	Kelly et al., 2009	*Naja naja*	AY713376	AY713378	AF435020	–
Ke11	Kelly et al., 2009	*Naja nivea*	AF217827	AY058983	AY058939	–
Ke12	Kelly et al., 2009	*Walterinnesia aegypta*	AF217838	AY058988	AY058943	–
Ke13	Kelly et al., 2009	*Ophiophagus hannah*	AF217842	AY058984	AY058940	–
Ke14	Kelly et al., 2009	*Amblyodipsas dimidiata*	DQ486346	DQ486322	DQ486170	–
Ke15	Kelly et al., 2009	*Homoroselaps lacteus*	AF217833	AY058976	AY058931	–
Ke16	Kelly et al., 2009	*Lamprophis fuliginosus*	DQ486339	DQ486315	DQ486163	–
Ke17	Kelly et al., 2009	*Prosymna ruspolii*	DQ486347	DQ486323	DQ486171	–
*# Ke18	Kelly et al., 2009	*Hemirhagerrhis kelleri*	DQ486335	DQ486311	DQ486159	–
*# Ke19	Kelly et al., 2009	*Malpolon monspessulanus*	AY058965	AY058989	AY058936	FJ404390
*# Ke20	Kelly et al., 2009	*Mimophis mahfalensis*	AY188032	AF544662	AY187993	FJ404391
*# Ke21	Kelly et al., 2009	*Psammophylax rhombeatus*	DQ486342	DQ486318	DQ486166	FJ404399
*# Ke22	Kelly et al., 2009	*Rhamphiophis rostratus*	DQ486336	DQ486312	DQ486160	FJ404401
Ke23	Kelly et al., 2009	*Pseudaspis cana*	DQ486343	DQ486319	DQ486167	–
Ke24	Kelly et al., 2009	*Leioheterodon modestus*	AY058967	AY058978	AY058933	–
Ke25	Kelly et al., 2009	*Amplorhinus multimaculatus*	DQ486340	DQ486316	DQ486164	–
Ke26	Kelly et al., 2009	*Buhoma procterae*	DQ486353	DQ486328	DQ486177	–
Ke27	Kelly et al., 2009	*Oxyrhabdium leporinum*	AF471029	–	DQ112081	–
Ke28	Kelly et al., 2009	*Acrochordus granulatus*	AF217841	U49296	AF471124	–
Ke29	Kelly et al., 2009	*Crotalus viridis*	AF471066	AF194157	AF471135	–
Ke30	Kelly et al., 2009	*Farancia abacura*	U69832	U49307	AF471141	–

Ke31	Kelly et al., 2009	*Contia tenius*				AF471095	AF258883	AF471134	–
Ke32	Kelly et al., 2009	*Diadophis punctatus*				AF471094	AF258910	AF471122	–
Ke33	Kelly et al., 2009	*Afronatrix anscopus*				AF420073	AF420076	AF471123	–
Ke34	Kelly et al., 2009	*Natrix natrix*				AF471059	AY873710	AF471121	–
Ke35	Kelly et al., 2009	*Cemophora coccinea*				AF471091	AF138754	AF471132	–
Ke36	Kelly et al., 2009	*Coronella girondica*				AF471088	AY487066	AF471113	–
Ke37	Kelly et al., 2009	*Hemorrhois algirus*				AY486911	AY487037	AY486935	–
Ke38	Kelly et al., 2009	*Hemorrhois hippocrepis*				AY486916	AY487045	AY486940	–
Ke39	Kelly et al., 2009	*Hemorrhois nummifer*				AY376742	AY487049	AY376800	–
Ke40	Kelly et al., 2009	*Hemorrhois ravergierei*				AY486920	AY487050	AY486944	–
Ke41	Kelly et al., 2009	*Grayia tholloni*				DQ486351	DQ486326	DQ486175	–
#V1	Vidal et al., 2007	*P. schokari*	Tunisia	34.5	9.5	AY612034	FJ404324	–	FJ404396
Wu01	Wuster et al., 2008	*Agkistrodon piscivorus*				AY223615	U41870	–	–
Wu02	Wuster et al., 2008	*Atheris squamigera*				EU624303	EU624212	–	–
Wu03	Wuster et al., 2008	*Atropoides picadoi*				AY223583	U41872	–	–
Wu04	Wuster et al., 2008	*Azemiops feae*				AY223559	U41865	–	–
Wu05	Wuster et al., 2008	*Bitis arietans*				EU624304	EU624213	–	–
Wu06	Wuster et al., 2008	*Bitis atropos*				AJ275691	EU624214	–	–
Wu07	Wuster et al., 2008	*Bitis gabonbica*				AJ275695	EU624217	–	–
Wu08	Wuster et al., 2008	*Bothriechis schlegelii*				AY223590	AY223636	–	–
Wu09	Wuster et al., 2008	*Bothrops alternatus*				AY223601	AY223642	–	–
Wu10	Wuster et al., 2008	*Calloselasma rhodostoma*				AY223562	U41878	–	–
Wu11	Wuster et al., 2008	*Cerastes cerastes*				EU624308	EU624222	–	–
Wu12	Wuster et al., 2008	*Crotalus adamanteus*				AY223605	U41880	–	–
Wu13	Wuster et al., 2008	*Crotalus ravus*				AY223609	AY223647	–	–
Wu14	Wuster et al., 2008	*Crotalus simus*				EU624302	AY704885	–	–
Wu15	Wuster et al., 2008	*Cryptelytrops insularis*				AY352767	AY352833	–	–
Wu16	Wuster et al., 2008	*Daboia mauritanica*				EU624313	EU624229	–	–
Wu18	Wuster et al., 2008	*Eirenis modestus*				AY486933	AY487072	–	–

Wu19	Wuster et al., 2008	*Eristicophis macmahonii*	AJ275711	EU624227	–	–
Wu20	Wuster et al., 2008	*Garthius chaseni*	AY352760	AY352825	–	–
Wu21	Wuster et al., 2008	*Lachesis stenophrys*	AY223603	U41885	–	–
Wu22	Wuster et al., 2008	*Macrovipera lebetina*	AJ275713	EU624228	–	–
Wu23	Wuster et al., 2008	*Montivipera xanthina*	AJ275724	EU624234	–	–
Wu24	Wuster et al., 2008	*Naja nigricollis*	EU624300	AY713377	–	–
Wu25	Wuster et al., 2008	*Ovophis monticola*	DQ305462	DQ305480	–	–
Wu26	Wuster et al., 2008	*Popeia popeiorum*	AY059571	AY059590	–	–
Wu27	Wuster et al., 2008	*Porthidium arcosae*	AF292575	AF292613	–	–
Wu28	Wuster et al., 2008	*Porthidium lansbergii*	AY713375	AF393623	–	–
Wu29	Wuster et al., 2008	*Porthidium nasutum*	AY223579	U41887	–	–
Wu30	Wuster et al., 2008	*Porthidium nasutum*	AF292574	AF292612	–	–
Wu31	Wuster et al., 2008	*Porthidium ophryomegas*	AY223580	U41888	–	–
Wu32	Wuster et al., 2008	*Proatheris superciliaris*	AJ275685	EU624230	–	–
Wu33	Wuster et al., 2008	*Sistrurus catenatus*	AY223610	AY223648	–	–
Wu34	Wuster et al., 2008	*Sistrurus miliarius*	AY223611	U41889	–	–
Wu35	Wuster et al., 2008	*Trimeresurus borneensis*	AY352754	AY352817	–	–
Wu36	Wuster et al., 2008	*Trimeresurus gracilis*	DQ305461	DQ305479	–	–
Wu37	Wuster et al., 2008	*Vipera ammodytes*	EU624314	EU624232	–	–
Wu38	Wuster et al., 2008	*Echis pyramidum*	EU624312	EU624226	–	–

[a]**BEV**: BEV collection, EPHE-CEFE-CNRS, France; **HDB**: "HerpetoDB", APPPHY, CIBIO-InBIO, Portugal; **JCB**: JC Brito's collection, CIBIO-InBIO, Portugal; **NHMC**: Natural History Museum of Crete; **SC.IBE**: Carranza Lab Collection, IBE, CSIC-UPF, Barcelona, Spain. Bibliographical references are given for the specimens whose sequences were obtained exclusively from GenBank.

Table A. 2 Climatic variables used in this work, depicting codes, names, units and ranges for both global and regional study areas. Variables highlighted in grey were used to model the distribution of *P. schokari* and its West African lineages. All variables for the regional study area were used to test niche overlap between Moroccan and Mauritanian lineages.

code	name	units	global	regional
BIO 1	Annual Mean Temperature	ºC	-15.5 - 31.9	8 - 30.8
BIO 2	Mean Diurnal Range	ºC	5.8 - 20.7	5.7 - 18.1
BIO 3	Isothermality	coefficient	21 - 84	27 - 71
BIO 4	Temperature Seasonality	coefficient	563 - 11124	1586 - 8767
BIO 5	Max Temperature of Warmest Month	ºC	2.9 - 48.9	22 - 49
BIO 6	Min Temperature of Coldest Month	ºC	-33.5 - 23	-15 - 17.2
BIO 7	Temperature Annual Range	ºC	11 - 47.8	10.9 - 42.9
BIO 10	Mean Temperature of Warmest Quarter	ºC	-4.1 - 38.2	10.4 - 37.9
BIO 11	Mean Temperature of Coldest Quarter	ºC	-27.6 - 27.6	-7.2 - 26.6
BIO 12	Annual Precipitation	mm	0 - 2769	4 - 1182
BIO 13	Precipitation of Wettest Month	mm	0 - 902	1 - 263
BIO 14	Precipitation of Driest Month	mm	0 - 42	0 - 29
BIO 16	Precipitation of Wettest Quarter	mm	0 - 2032	3 - 690
BIO 19	Precipitation of Coldest Quarter	mm	0 - 1185	0 - 573

Table A. 3 Best-fit partitioning schemes and models, according to PARTITIONFINDER, and characteristics of each alignment. Numbers in front of gene names indicate codon positions. N: number of samples with data; Length: length of marker in base-pairs; V: variable (polymorphic) sites; Pi: parsimony informative sites. V and Pi were calculated in MEGA6.

Dataset	Initial partition	Subset Partitions	Best Model	N, Length, V, Pi
1	By gene	CYTB, ND4	GTR+I+G	ND4: 95, 696, 319, 258
		c-mos, RAG2	HKY+I+G	CYTB: 91, 1113, 496, 389
	By codon	CYTB_1, ND4_1	GTR+I+G	c-mos: 81, 588, 75, 17
		CYTB_2, ND4_2, RAG2_3	HKY+I+G	RAG2: 74, 714, 60, 26
		CYTB_3, ND4_3	GTR+I+G	
		c-mos_1, c-mos_2, RAG2_1, RAG2_2	HKY+I+G	
		c-mos_3	HKY	
2	By gene	CYTB, ND4	GTR+I+G	ND4: 124, 696, 466, 415
		c-mos	HKY+G	CYTB: 125, 1116, 703, 624
	By codon	CYTB_1, ND4_1	GTR+I+G	c-mos: 65, 567, 199, 107
		CYTB_2, ND4_2	GTR+I+G	
		CYTB_3, ND4_3	GTR+I+G	
		c-mos_1, c-mos_2	K80+G	
		c-mos_3	HKY+I	

Table A. 4. CONSEL topology test results. AU: Approximately Unbiased test (Shimodaira, 2002); SH: Shimodaira & Hasegawa (1999) test; pp: posterior probability calculated from the log-likelihood values. Values < 0.05 suggest topologies are significantly different.

Topology	-log likelihood	AU *P*	pp	SH *P*
Africa paraphyletic	20403.429540	0.618	0.367	0.633
Africa monophyletic	20405.700511	0.382	0.094	0.367

AU: Approximately Unbiased test (Shimodaira, 2002); SH: Shimodaira and Hasegawa (1999) test; pp: posterior probability calculated from the log-likelihood values. Values < 0.05 suggest topologies are significantly different.

Appendix B – Supplementary material of chapter 5.2

Text B1. The case of *Agama boulengeri*.

The geographic area with available genetic information was significantly increased for species and lineages in comparison with previous studies (Gonçalves et al., 2012; Mediannikov et al., 2012; Leaché et al., 2014). The number of known major intra-specific genetic lineages has also increased, particularly regarding *A. boulengeri* (Gonçalves et al., 2012).

Agama boulengeri displays a rather complex pattern. The four main mtDNA lineages have clear parapatric distributions, with two potential barriers or contact zones: Karakoro River and Djouk valleys. These valleys correspond to unsuitable habitat, but aridification and sand encroaching are not enough to explain the pattern due to two reasons: first, the sandy area separating the Tagant and Adrar-Atar mountains, much larger than Djouk valley, is within the distribution of the N lineage (Vale et al., 2012) and results in no apparent gene-flow break; second, the earliest split within *A. boulengeri* was between S lineage (the three bPTP S lineages) and the remaining ones, but N and S lineages are the closest geographically. Then there is the discordance of the nuclear haplotype distribution and the phylogenetic tree. This pattern could be dismissible as nuclear incomplete lineage sorting, but there are also two points to consider: first, given the crown age is around 6Ma (Miocene), nucleotide diversity is lower and haplotype sharing larger than expected based on the other species; second, the E lineage is the one most separated in terms of nuclear haplotypes, but it originates from the third oldest split (Fig. 2). Therefore, the nuclear haplotype sharing could likewise be the result of secondary contact or hybridization among species. If each of the four main lineages had diversified in allopatry and then came into contact, the observed pattern would be coherent with the landscape: the N and S populations would have more gene-flow between them, and so have higher allele sharing, while the E population, being separated from these two by a wider patch of unsuitable habitat, would have less allele sharing with them. Nevertheless, with only two slow evolving nuclear markers, the incomplete lineage sorting cannot be ruled out, even considering the high number of samples. Also, with such a high level of admixture

between N and S, such a well-defined border between mtDNA lineages is unexpected. It seems that aridification, through range contractions or sand encroaching, may have played a role in the diversification of *A. boulengeri*, but a more detailed assessment of gene-flow and the role of landscape will be needed to properly clarify the evolution of the species. Strictly considering the bPTP delimitation, four lineages (N plus the 3 comprising S) occur in Djouk Valley, making it a diversity hotspot and likely *refugia* for the species.

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Text B2. Notes on equivalency tests.

This text addresses the differences in the calculation and meaning of p-values for equivalency tests, between the R package ECOSPAT (Broennimann et al., 2016) and the previous scripts from Broennimann et al scripts (2012).

In the older scripts the value was obtained by:

```
min((sum(sim.o$D<=obs.o$D)+1),(sum(sim.o$D>=obs.o$D)+1))*2/(length(sim.o$D)+1)
```

therefore testing if the observed values were significantly inside or outside the simulated distribution (Warren et al., 2008), but never going above 0.5 (since it was always the smaller section of the simulated distribution that was reported). In the ECOSPAT function, and the present work, equivalency tests have two “alternatives” (greater and lower, for testing for niche conservatism and divergence, respectively), and e.g. if alternative == "greater" p-values are obtained by:

```
(sum(sim.o$D>=obs.o$D)+1)/(length(sim.o$D)+1),
```

thus ranging from 0 to 1, but this time being a directional test.

Previous studies have reported very low values for equivalency tests (Rato et al., 2015; Ahmadzadeh et al., 2016; Martínez-Freiría et al., 2017; Gonçalves et al., 2018), and here we have obtained very high values. However, if subject to the previous methodology the p-values here obtained (1 or near) would be close to 0, thus following the same pattern as other published works.

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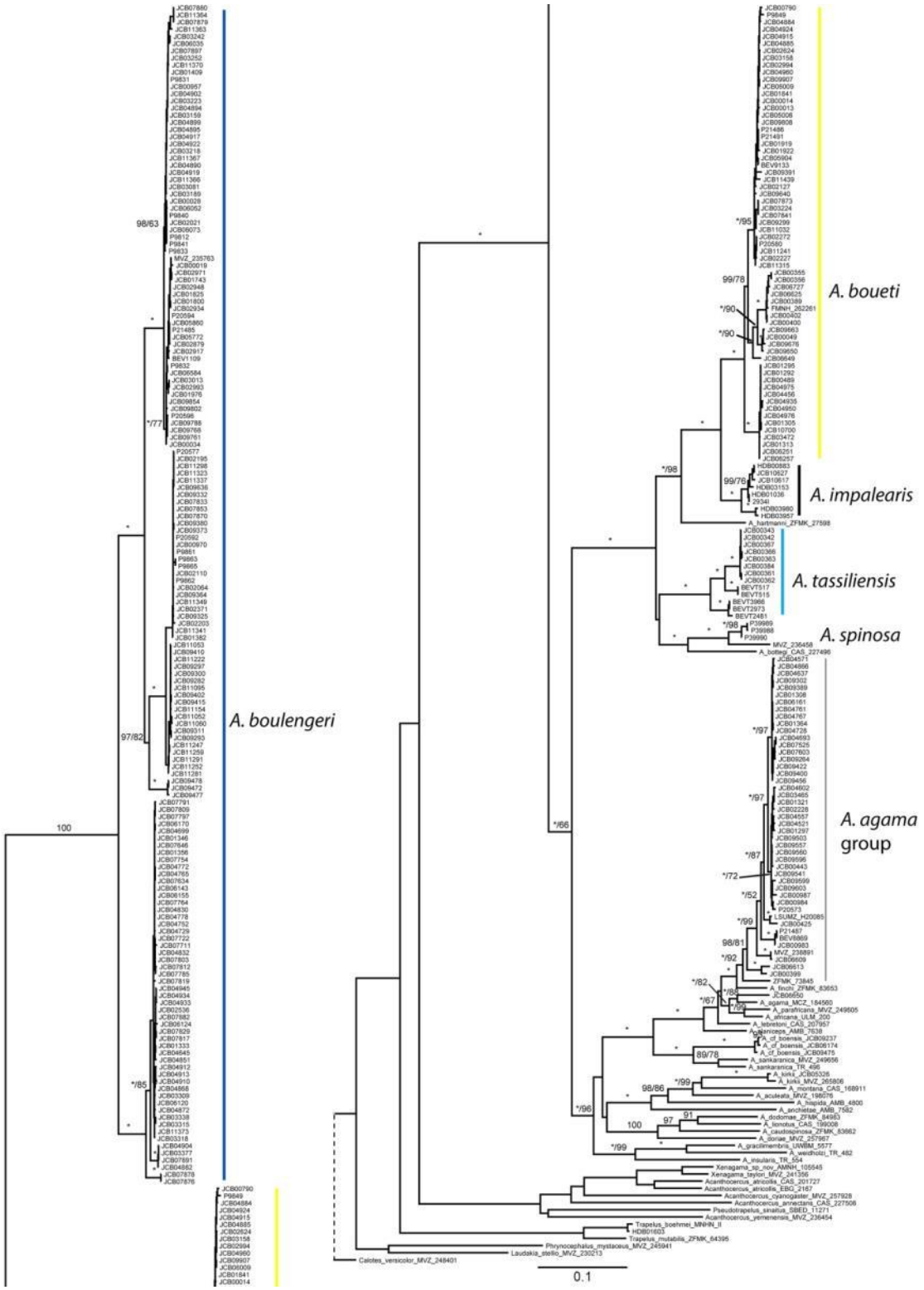

Figure B. 1. Phylogenetic relationships among *Agama* species, including the samples with unique concatenated sequences of ND4+tRNAs, 16S, NT3 and c-mos. The represented tree was obtained in RAxML. Support values next to nodes indicate Bayesian posterior probability (bpp) from MrBayes (%) and bootstrap support (bss) from RAxML (bpp/bss); asterisks represent values of 100. Support values for terminal nodes and the full length of the tree root, were not represented to improve clarity; species and lineages (capital letters, see also Figure B2) are indicated next to the samples.

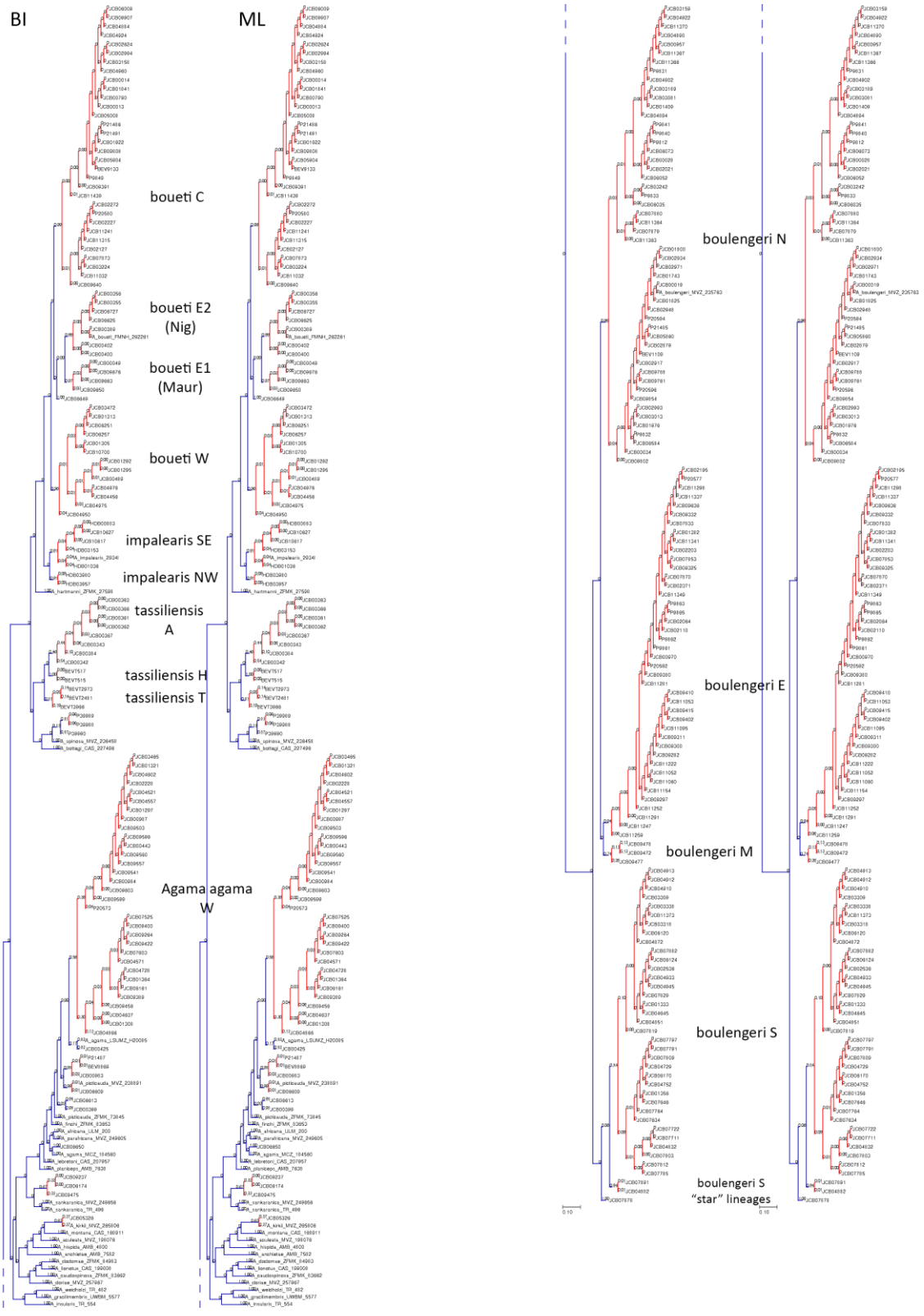

Figure B. 2. bPTP lineage delimitation for the Bayesian (MrBayes) tree (left), and Maximum-likelihood (RAxML) tree (right). Outgroups were not included, in accordance to the program's guidelines. Each connected group of red branches indicate a lineage. Correspondent species and lineages are indicated between the trees.

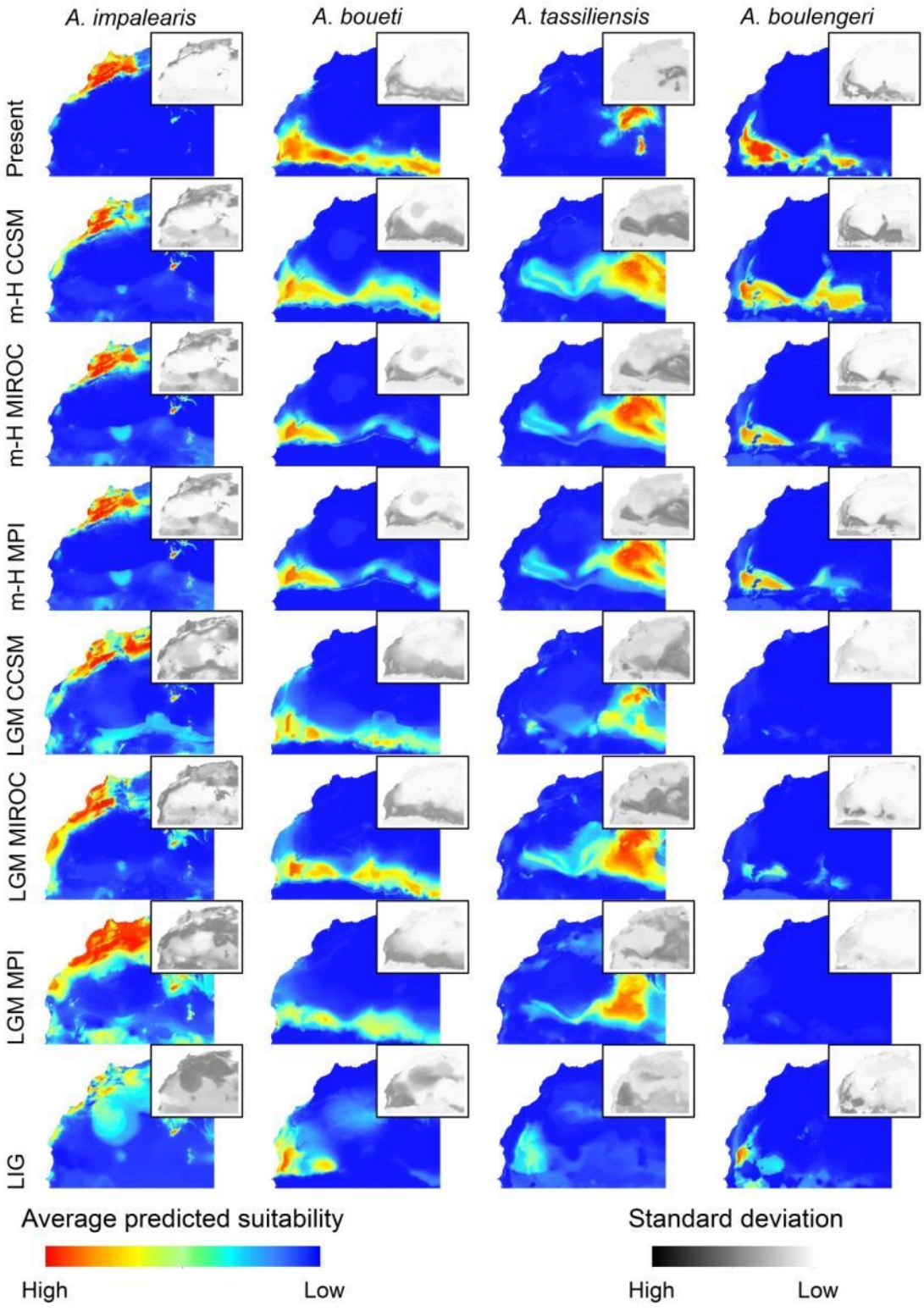

Figure B. 3. Probabilistic models for current conditions and respective projections to past conditions (LIG, LGM and Mid-Holocene) calculated for *A. impalearis*, *A. boueti*, *A. tassiliensis*, and *A. boulengeri* and using BIOMOD2. Each map corresponds to the ensemble forecast of four modelling algorithms (ANN, GAM, GLM and MAXENT).

Table B. 1. List of genetic samples used in this study, including from GenBank (see footnote). Coordinates are in decimal degrees in the WGS84 datum.

	Sample	Species	Lat.	Long.	16S	ND4	c-mos	NT3
G *	MVZ.198076	*A. aculeata*			GU128430	GU128467	JX838901	JX839185
G *	ULM.200	*A. africana*			GU128440	GU128477	JX838902	JX839186
G#*	LSUMZ.H20085	*A. agama*	8.789	-1.468	GU128438	GU128475	–	–
G *	MCZ.184560	*A. agama*	3.895	11.513	JX668144	JX857595	JX838903	JX839187
G *	AMB.7582	*A. anchietae*			GU128446	GU128483	JX838905	JX839189
G *	CAS.227496	*A. bottegi*			JX668157	JX857587	JX838916	JX839199
G#*	FMNH.262261	*A. boueti*			JX668160	JX857623	JX838919	JX839202
G#*	MVZ.235763	*A. boulengeri*	20.565	-13.105	JX668163	JX857603	JX838923	JX839206
G *	ZFMK.83662	*A. caudospinosa*			GU128450	GU128487	JX838926	–
G *	ZFMK.84983	*A. dodomae*			JX668167	JX857552	JX838927	–
G *	MVZ.257967	*A. doriae*			JX668168	JX857614	JX838928	JX839207
G *	ZFMK.83653	*A. finchi*	0.636	34.276	GU128452	GU128489	JX838933	–
G *	UWBM.5577	*A. gracilimembris*			JX668174	JX857563	JX838935	–
G *	ZFMK.27598	*A. hartmanni*			JX668176	JX857590	–	–
G *	AMB.4800	*A. hispida*			GU128453	GU128490	JX838937	JX839208
G#*	2934I	*A. impalearis*			JX668178	JX857625	JX838939	JX839210
G *	TR 554	*A. insularis*			JX668181	JX857550	JX838941	JX839212
G *	MVZ.265806	*A. kirkii*			JX668184	JX857624	JX838944	–
G *	CAS.207957	*A. lebretoni*			JX668192	JX857627	JX838953	JX839219
G *	CAS.199008	*A. lionotus*			JX668193	JX857597	JX838955	–
G *	CAS.168911	*A. montana*			JX668198	JX857579	JX838959	JX839223
G *	MVZ.249605	*A. parafricana*			JX668201	JX857612	JX838962	JX839225
G#*	MVZ.238891	*A. agama*	13.504	2.114	GU128443	GU128480	JX838965	JX839228
G#*	ZFMK.73845	*A. agama*	14.769	-17.301	JX668204	JX857605	JX838967	–
G *	AMB.7638	*A. planiceps*			GU128458	GU128494	JX838970	JX839232
G *	MVZ.249656	*A. sankaranica*			GU128460	GU128496	JX838976	–
G *	TR.496	*A. sankaranica*			JX668156	JX857575	JX838915	–
G *	MVZ.236458	*A. spinosa*			GU128461	GU128497	JX838977	JX839238
G *	TR.482	*A. weidholzi*			JX668216	JX857554	JX838983	JX839244
G *	CAS.227508	*Acanthocercus annectans*			JX668128	JX857621	JX838886	JX839175
G *	CAS.201727	*Acanthocercus atricollis*			JX668130	JX857596	JX838888	JX839177
G *	EBG.2167	*Acanthocercus atricollis*			JX668132	JX857631	JX838890	JX839179
G *	MVZ.257928	*Acanthocercus cyanogaster*			JX668137	JX857548	JX838894	JX839180
G *	MVZ.236454	*Acanthocercus yemenensis*			JX668140	JX857608	JX838897	JX839181
G *	MVZ.248401	*Calotes versicolor*			JX668217	JX857560	JX838985	JX839246
G *	MVZ.230213	*Laudakia stellio*			GU128464	GU128500	JX838986	JX839247
G *	MVZ.245941	*Phrynocephalus mystaceus*			JX668218	JX857553	JX838987	JX839248
G *	SBED.11271	*Pseudotrapelus sinaitus*			JX668219	JX857606	JX838988	JX839249
G *	MNHN.II	*Trapelus boehmei*			JX668221	JX857619	JX838989	JX839250
G *	ZFMK.64395	*Trapelus mutabilis*			HQ901114	GU128501	–	–
G *	AMNH.105545	*Xenagama sp.*			JX668225	JX857585	JX838991	JX839251
G *	MVZ.241356	*Xenagama taylori*			GU128466	GU128502	JX838993	JX839253
#*	BEV01109	*A. boulengeri*	20.927	-11.624	pending	pending	pending	–
#*	BEV08869	*A. agama*	14.533	-4.100	pending	pending	pending	–
#*	BEV09133	*A. boueti*	21.154	-11.910	JN665060	pending	pending	–

#*	BEVT00515	*A. tassiliensis*	23.126	5.989	pending	pending	pending	pending
#*	BEVT00517	*A. tassiliensis*	23.479	5.781	JN665063	pending	pending	pending
#*	BEVT02481	*A. tassiliensis*	24.741	9.187	pending	pending	pending	–
#*	BEVT02973	*A. tassiliensis*	25.350	8.391	JN665061	pending	pending	–
#*	BEVT03966	*A. tassiliensis*	24.869	8.826	JN665062	pending	pending	–
#*	HDB00883	*A. impalearis*	28.269	-11.211	pending	pending	pending	pending
#*	HDB01036	*A. impalearis*	30.980	-5.822	pending	pending	pending	–
#*	HDB01603	*Trapelus boehmei*	29.110	-9.140	pending	pending	pending	pending
#*	HDB03153	*A. impalearis*	32.530	-1.017	pending	pending	pending	pending
#*	HDB03957	*A. impalearis*	35.063	-5.195	pending	pending	pending	pending
#*	HDB03980	*A. impalearis*	33.212	-8.551	pending	pending	pending	pending
#*	JCB00013	*A. boueti*	20.144	-12.547	pending	pending	pending	pending
#*	JCB00014	*A. boueti*	20.108	-12.464	pending	pending	pending	pending
#*	JCB00019	*A. boulengeri*	18.877	-11.819	pending	pending	pending	pending
#*	JCB00028	*A. boulengeri*	18.553	-11.434	pending	pending	pending	–
#*	JCB00034	*A. boulengeri*	18.536	-10.405	pending	pending	pending	pending
#*	JCB00049	*A. boueti*	17.407	-7.222	pending	pending	pending	pending
#*	JCB00342	*A. tassiliensis*	16.952	8.674	pending	pending	pending	pending
#*	JCB00343	*A. tassiliensis*	16.952	8.674	JX128146	pending	pending	pending
#*	JCB00355	*A. boueti*	17.522	8.667	pending	pending	pending	pending
#*	JCB00356	*A. boueti*	17.522	8.667	pending	pending	pending	pending
#*	JCB00361	*A. tassiliensis*	17.986	8.765	pending	pending	pending	pending
#*	JCB00362	*A. tassiliensis*	17.986	8.765	JX128150	pending	pending	pending
#*	JCB00363	*A. tassiliensis*	17.986	8.765	JX128151	pending	pending	pending
#*	JCB00366	*A. tassiliensis*	17.998	8.754	pending	pending	pending	pending
#*	JCB00367	*A. tassiliensis*	18.013	8.741	pending	pending	pending	pending
#*	JCB00384	*A. tassiliensis*	18.098	8.767	pending	pending	pending	pending
#*	JCB00389	*A. boueti*	17.711	8.274	pending	pending	pending	pending
#*	JCB00399	*A. agama*	14.509	5.377	pending	pending	pending	pending
#*	JCB00400	*A. boueti*	13.786	5.013	pending	pending	pending	pending
#*	JCB00402	*A. boueti*	13.787	4.969	pending	pending	pending	pending
#*	JCB00425	*A. agama*	12.102	0.248	pending	pending	pending	pending
#*	JCB00443	*A. agama*	14.522	-9.550	pending	pending	pending	pending
#*	JCB00489	*A. boueti*	17.393	-16.062	pending	pending	pending	pending
#*	JCB00790	*A. boueti*	19.921	-12.926	pending	pending	pending	pending
#*	JCB00957	*A. boulengeri*	17.872	-11.606	pending	pending	pending	pending
#*	JCB00970	*A. boulengeri*	16.507	-10.415	pending	pending	pending	pending
#*	JCB00983	*A. agama*	14.610	-3.201	pending	pending	pending	pending
#*	JCB00984	*A. agama*	14.495	-4.201	pending	pending	pending	pending
#*	JCB00987	*A. agama*	13.659	-5.483	pending	pending	pending	pending
#*	JCB01292	*A. boueti*	16.827	-15.348	pending	pending	pending	pending
#*	JCB01295	*A. boueti*	16.891	-15.203	pending	pending	pending	–
#*	JCB01297	*A. agama*	16.891	-15.203	pending	pending	pending	pending
#*	JCB01305	*A. boueti*	16.750	-14.734	pending	pending	pending	pending
#*	JCB01308	*A. agama*	16.415	-14.081	pending	pending	pending	pending
#*	JCB01313	*A. boueti*	16.301	-13.875	pending	pending	pending	pending
#*	JCB01321	*A. agama*	16.173	-13.020	pending	pending	pending	pending
#*	JCB01333	*A. boulengeri*	15.971	-12.685	pending	pending	pending	pending

#*	JCB01346	*A. boulengeri*	15.883	-12.036	pending	pending	pending	pending
#*	JCB01356	*A. boulengeri*	15.902	-11.826	pending	pending	pending	pending
#*	JCB01364	*A. agama*	16.077	-11.512	pending	pending	pending	pending
#*	JCB01382	*A. boulengeri*	16.640	-11.056	pending	pending	pending	pending
#*	JCB01409	*A. boulengeri*	17.274	-12.227	pending	pending	pending	–
#*	JCB01743	*A. boulengeri*	20.253	-13.088	pending	pending	pending	pending
#*	JCB01800	*A. boulengeri*	19.722	-12.866	pending	pending	pending	pending
#*	JCB01825	*A. boulengeri*	19.525	-12.961	pending	pending	pending	pending
#*	JCB01841	*A. boueti*	19.764	-13.082	pending	pending	pending	pending
#*	JCB01919	*A. boueti*	20.243	-12.676	pending	pending	pending	pending
#*	JCB01922	*A. boueti*	20.146	-12.549	pending	pending	pending	pending
#*	JCB01976	*A. boulengeri*	18.687	-11.617	pending	pending	pending	pending
#*	JCB02021	*A. boulengeri*	18.014	-11.588	pending	pending	pending	pending
#*	JCB02064	*A. boulengeri*	17.102	-10.956	pending	pending	pending	pending
#*	JCB02110	*A. boulengeri*	17.032	-10.245	pending	pending	pending	–
#*	JCB02127	*A. boueti*	17.010	-10.171	pending	pending	pending	pending
#*	JCB02195	*A. boulengeri*	16.864	-9.583	pending	pending	pending	pending
#*	JCB02203	*A. boulengeri*	16.428	-9.562	pending	pending	pending	pending
#*	JCB02227	*A. boueti*	16.468	-10.029	pending	pending	pending	pending
#*	JCB02228	*A. agama*	16.383	-10.287	pending	pending	pending	pending
#*	JCB02272	*A. boueti*	16.702	-10.184	pending	pending	pending	pending
#*	JCB02371	*A. boulengeri*	16.579	-10.705	pending	pending	pending	pending
#*	JCB02536	*A. boulengeri*	16.547	-12.010	pending	pending	pending	pending
#*	JCB02624	*A. boueti*	17.402	-12.389	pending	pending	pending	pending
#*	JCB02879	*A. boulengeri*	21.274	-11.933	pending	pending	pending	pending
#*	JCB02917	*A. boulengeri*	20.003	-13.270	pending	pending	pending	pending
#*	JCB02934	*A. boulengeri*	19.762	-13.427	pending	pending	pending	pending
#*	JCB02948	*A. boulengeri*	19.083	-13.010	pending	pending	pending	pending
#*	JCB02971	*A. boulengeri*	18.584	-12.657	pending	pending	pending	pending
#*	JCB02993	*A. boulengeri*	18.312	-12.234	pending	pending	pending	pending
#*	JCB02994	*A. boueti*	18.312	-12.234	pending	pending	pending	pending
#*	JCB03013	*A. boulengeri*	18.436	-12.043	pending	pending	pending	pending
#*	JCB03081	*A. boulengeri*	17.762	-11.883	pending	pending	pending	pending
#*	JCB03158	*A. boueti*	17.667	-12.197	pending	pending	pending	pending
#*	JCB03159	*A. boulengeri*	17.667	-12.197	pending	pending	pending	pending
#*	JCB03189	*A. boulengeri*	17.241	-12.102	pending	pending	pending	pending
#*	JCB03218	*A. boulengeri*	17.398	-12.031	pending	pending	pending	–
#*	JCB03223	*A. boulengeri*	17.373	-11.916	pending	pending	pending	pending
#*	JCB03224	*A. boueti*	17.373	-11.916	pending	pending	pending	pending
#*	JCB03242	*A. boulengeri*	17.388	-11.612	pending	pending	pending	pending
#*	JCB03252	*A. boulengeri*	17.173	-11.938	pending	pending	pending	pending
#*	JCB03309	*A. boulengeri*	16.787	-11.921	pending	pending	pending	pending
#*	JCB03315	*A. boulengeri*	16.830	-11.834	pending	pending	pending	pending
#*	JCB03318	*A. boulengeri*	16.877	-11.964	pending	pending	pending	pending
#*	JCB03338	*A. boulengeri*	16.988	-12.047	pending	pending	pending	pending
#*	JCB03377	*A. boulengeri*	17.152	-12.199	pending	pending	pending	pending
#*	JCB03465	*A. agama*	16.904	-12.820	pending	pending	pending	pending
#*	JCB03472	*A. boueti*	16.773	-13.210	pending	pending	pending	pending

#*	JCB04456	*A. boueti*	16.924	-16.318	pending	pending	pending	pending
#*	JCB04521	*A. agama*	16.600	-15.765	pending	pending	pending	pending
#*	JCB04557	*A. agama*	16.744	-15.367	pending	pending	pending	pending
#*	JCB04571	*A. agama*	16.650	-14.412	pending	pending	pending	pending
#*	JCB04602	*A. agama*	16.147	-13.519	pending	pending	pending	pending
#*	JCB04637	*A. agama*	15.967	-12.678	pending	pending	pending	pending
#*	JCB04645	*A. boulengeri*	15.731	-12.807	pending	pending	pending	pending
#*	JCB04693	*A. agama*	15.216	-12.233	pending	pending	pending	pending
#*	JCB04699	*A. boulengeri*	15.149	-12.152	pending	pending	pending	pending
#*	JCB04728	*A. agama*	15.591	-11.880	pending	pending	pending	pending
#*	JCB04729	*A. boulengeri*	15.591	-11.880	pending	pending	pending	pending
#*	JCB04752	*A. boulengeri*	15.581	-11.974	pending	pending	pending	pending
#*	JCB04761	*A. agama*	15.682	-12.163	pending	pending	pending	pending
#*	JCB04765	*A. boulengeri*	15.682	-12.163	pending	pending	pending	pending
#*	JCB04767	*A. agama*	15.682	-12.163	pending	pending	pending	pending
#*	JCB04772	*A. boulengeri*	15.773	-12.200	pending	pending	pending	pending
#*	JCB04778	*A. boulengeri*	15.929	-12.218	pending	pending	pending	pending
#*	JCB04830	*A. boulengeri*	16.066	-12.072	pending	pending	pending	pending
#*	JCB04832	*A. boulengeri*	16.196	-12.043	pending	pending	pending	pending
#*	JCB04851	*A. boulengeri*	16.593	-12.144	pending	pending	pending	pending
#*	JCB04866	*A. agama*	16.723	-12.180	pending	pending	pending	pending
#*	JCB04868	*A. boulengeri*	16.959	-12.312	pending	pending	pending	pending
#*	JCB04872	*A. boulengeri*	17.068	-12.260	pending	pending	pending	–
#*	JCB04882	*A. boulengeri*	17.163	-12.179	pending	pending	pending	pending
#*	JCB04884	*A. boueti*	17.166	-12.174	pending	pending	pending	pending
#*	JCB04885	*A. boueti*	17.169	-12.173	pending	pending	pending	pending
#*	JCB04890	*A. boulengeri*	17.173	-12.172	pending	pending	pending	pending
#*	JCB04894	*A. boulengeri*	17.189	-12.160	pending	pending	pending	pending
#*	JCB04895	*A. boulengeri*	17.178	-12.130	pending	pending	pending	pending
#*	JCB04899	*A. boulengeri*	17.162	-12.113	pending	pending	pending	pending
#*	JCB04902	*A. boulengeri*	17.144	-12.123	pending	pending	pending	pending
#*	JCB04904	*A. boulengeri*	17.158	-12.143	pending	pending	pending	pending
#*	JCB04910	*A. boulengeri*	17.177	-12.219	pending	pending	pending	pending
#*	JCB04912	*A. boulengeri*	17.200	-12.258	pending	pending	pending	pending
#*	JCB04913	*A. boulengeri*	17.200	-12.258	pending	pending	pending	pending
#*	JCB04915	*A. boueti*	17.256	-12.283	pending	pending	pending	pending
#*	JCB04917	*A. boulengeri*	17.258	-12.284	pending	pending	pending	pending
#*	JCB04919	*A. boulengeri*	17.288	-12.276	pending	pending	pending	pending
#*	JCB04922	*A. boulengeri*	17.246	-12.218	pending	pending	pending	pending
#*	JCB04924	*A. boueti*	17.200	-12.432	pending	pending	pending	pending
#*	JCB04933	*A. boulengeri*	17.094	-12.499	pending	pending	pending	pending
#*	JCB04934	*A. boulengeri*	17.102	-12.536	pending	pending	pending	pending
#*	JCB04935	*A. boueti*	17.111	-12.608	pending	pending	pending	pending
#*	JCB04945	*A. boulengeri*	17.070	-12.689	pending	pending	pending	pending
#*	JCB04950	*A. boueti*	17.070	-12.689	pending	pending	pending	pending
#*	JCB04960	*A. boueti*	17.574	-12.901	pending	pending	pending	pending
#*	JCB04975	*A. boueti*	17.391	-13.456	pending	pending	pending	pending
#*	JCB04976	*A. boueti*	17.344	-13.614	pending	pending	pending	pending

#*	JCB05008	*A. boueti*	19.792	-14.278	pending	pending	pending	pending
#*	JCB05326	*A. sp.*	-14.037	34.826	pending	pending	pending	pending
#*	JCB05772	*A. boulengeri*	21.526	-12.864	pending	pending	pending	pending
#*	JCB05860	*A. boulengeri*	20.554	-12.692	pending	pending	pending	pending
#*	JCB05904	*A. boueti*	21.152	-11.947	pending	pending	pending	pending
#*	JCB06009	*A. boueti*	17.659	-12.627	pending	pending	pending	pending
#*	JCB06035	*A. boulengeri*	18.053	-11.943	pending	pending	pending	pending
#*	JCB06052	*A. boulengeri*	18.258	-11.513	pending	pending	pending	pending
#*	JCB06073	*A. boulengeri*	17.650	-11.395	pending	pending	pending	pending
#*	JCB06120	*A. boulengeri*	16.723	-12.289	pending	pending	pending	pending
#*	JCB06124	*A. boulengeri*	16.406	-12.499	pending	pending	pending	pending
#*	JCB06143	*A. boulengeri*	15.825	-12.530	pending	pending	pending	pending
#*	JCB06155	*A. boulengeri*	15.692	-12.471	pending	pending	pending	pending
#*	JCB06161	*A. agama*	15.635	-12.433	pending	pending	pending	pending
#*	JCB06170	*A. boulengeri*	15.402	-12.236	pending	pending	pending	pending
#*	JCB06174	*A. boensis*	14.993	-12.319	pending	pending	pending	pending
#*	JCB06251	*A. boueti*	15.626	-13.147	pending	pending	pending	pending
#*	JCB06257	*A. boueti*	15.742	-13.240	pending	pending	pending	pending
#*	JCB06584	*A. boulengeri*	18.094	-12.131	pending	pending	pending	pending
#*	JCB06609	*A. agama*	13.529	2.052	pending	pending	pending	pending
#*	JCB06613	*A. agama*	13.803	8.979	pending	pending	pending	pending
#*	JCB06625	*A. boueti*	13.708	9.373	pending	pending	pending	pending
#*	JCB06649	*A. boueti*	13.511	12.823	pending	pending	pending	pending
#*	JCB06650	*A. paragama*	13.511	12.823	pending	pending	pending	pending
#*	JCB06727	*A. boueti*	15.867	11.455	pending	pending	pending	pending
#*	JCB07525	*A. agama*	15.088	-12.615	pending	pending	pending	pending
#*	JCB07603	*A. agama*	14.799	-12.221	pending	pending	pending	pending
#*	JCB07634	*A. boulengeri*	15.591	-12.243	pending	pending	pending	pending
#*	JCB07646	*A. boulengeri*	15.957	-12.010	pending	pending	pending	pending
#*	JCB07711	*A. boulengeri*	16.339	-11.978	pending	pending	pending	pending
#*	JCB07722	*A. boulengeri*	16.297	-12.005	pending	pending	pending	pending
#*	JCB07754	*A. boulengeri*	16.003	-11.872	pending	pending	pending	pending
#*	JCB07764	*A. boulengeri*	15.945	-11.929	pending	pending	pending	pending
#*	JCB07785	*A. boulengeri*	15.949	-11.682	pending	pending	pending	pending
#*	JCB07791	*A. boulengeri*	16.031	-11.619	pending	pending	pending	pending
#*	JCB07797	*A. boulengeri*	16.053	-11.670	pending	pending	pending	pending
#*	JCB07803	*A. boulengeri*	16.108	-11.708	pending	pending	pending	pending
#*	JCB07809	*A. boulengeri*	16.163	-11.748	pending	pending	pending	pending
#*	JCB07812	*A. boulengeri*	16.444	-11.778	pending	pending	pending	pending
#*	JCB07817	*A. boulengeri*	16.560	-11.724	pending	pending	pending	pending
#*	JCB07819	*A. boulengeri*	16.530	-11.807	pending	pending	pending	pending
#*	JCB07829	*A. boulengeri*	16.619	-11.846	pending	pending	pending	pending
#*	JCB07833	*A. boulengeri*	16.663	-11.345	pending	pending	pending	pending
#*	JCB07841	*A. boueti*	16.692	-11.282	pending	pending	pending	pending
#*	JCB07853	*A. boulengeri*	16.778	-11.144	pending	pending	pending	pending
#*	JCB07870	*A. boulengeri*	16.765	-11.269	pending	pending	pending	pending
#*	JCB07873	*A. boueti*	16.802	-11.397	pending	pending	pending	pending
#*	JCB07876	*A. boulengeri*	17.130	-11.523	pending	pending	pending	pending

#*	JCB07878	*A. boulengeri*	17.130	-11.523	pending	pending	pending	pending
#*	JCB07879	*A. boulengeri*	16.871	-11.709	pending	pending	pending	pending
#*	JCB07880	*A. boulengeri*	16.871	-11.709	pending	pending	pending	pending
#*	JCB07882	*A. boulengeri*	16.905	-11.886	pending	pending	pending	pending
#*	JCB07891	*A. boulengeri*	17.125	-12.094	pending	pending	pending	pending
#*	JCB07897	*A. boulengeri*	17.135	-12.090	pending	pending	pending	pending
#*	JCB09237	*A. boensis*	15.096	-12.244	pending	pending	pending	pending
#*	JCB09264	*A. agama*	15.118	-11.848	pending	pending	pending	pending
#*	JCB09282	*A. boulengeri*	15.895	-11.452	pending	pending	pending	pending
#*	JCB09293	*A. boulengeri*	15.924	-11.274	pending	pending	pending	pending
#*	JCB09297	*A. boulengeri*	15.958	-11.106	pending	pending	pending	pending
#*	JCB09299	*A. boueti*	16.015	-11.053	pending	pending	pending	pending
#*	JCB09300	*A. boulengeri*	16.015	-11.053	pending	pending	pending	pending
#*	JCB09302	*A. agama*	16.064	-11.063	pending	pending	pending	pending
#*	JCB09311	*A. boulengeri*	16.161	-10.944	pending	pending	pending	pending
#*	JCB09325	*A. boulengeri*	16.262	-11.053	pending	pending	pending	pending
#*	JCB09332	*A. boulengeri*	16.423	-11.007	pending	pending	–	pending
#*	JCB09364	*A. boulengeri*	16.490	-11.058	pending	pending	–	pending
#*	JCB09373	*A. boulengeri*	16.460	-11.158	pending	pending	pending	pending
#*	JCB09380	*A. boulengeri*	16.403	-11.262	pending	pending	pending	pending
#*	JCB09389	*A. agama*	16.386	-11.376	pending	pending	pending	pending
#*	JCB09391	*A. boueti*	16.313	-11.486	pending	pending	pending	pending
#*	JCB09400	*A. agama*	15.377	-11.598	pending	pending	pending	pending
#*	JCB09402	*A. boulengeri*	15.213	-11.628	pending	pending	pending	pending
#*	JCB09410	*A. boulengeri*	15.130	-11.643	pending	pending	pending	pending
#*	JCB09415	*A. boulengeri*	15.037	-11.622	pending	pending	pending	pending
#*	JCB09422	*A. agama*	14.843	-11.504	pending	pending	pending	pending
#*	JCB09456	*A. agama*	14.472	-11.510	pending	pending	pending	pending
#*	JCB09472	*A. boulengeri*	14.364	-11.359	pending	pending	pending	pending
#*	JCB09475	*A. boensis*	14.372	-11.377	pending	pending	pending	pending
#*	JCB09477	*A. boulengeri*	14.365	-11.375	pending	pending	pending	pending
#*	JCB09478	*A. boulengeri*	14.365	-11.375	pending	pending	pending	pending
#*	JCB09503	*A. agama*	14.109	-11.023	pending	pending	pending	pending
#*	JCB09541	*A. agama*	14.320	-10.413	pending	pending	pending	pending
#*	JCB09557	*A. agama*	14.656	-10.288	pending	pending	pending	pending
#*	JCB09560	*A. agama*	14.504	-9.875	pending	pending	pending	pending
#*	JCB09596	*A. agama*	14.918	-9.364	pending	pending	pending	pending
#*	JCB09599	*A. agama*	15.237	-9.567	pending	pending	pending	pending
#*	JCB09603	*A. agama*	15.940	-9.457	pending	pending	pending	pending
#*	JCB09636	*A. boulengeri*	16.449	-9.247	pending	pending	pending	pending
#*	JCB09640	*A. boueti*	16.411	-8.927	pending	pending	pending	pending
#*	JCB09650	*A. boueti*	16.481	-7.675	pending	pending	pending	pending
#*	JCB09663	*A. boueti*	17.057	-7.300	pending	pending	pending	pending
#*	JCB09676	*A. boueti*	17.472	-7.424	pending	pending	pending	pending
#*	JCB09761	*A. boulengeri*	18.397	-8.850	pending	pending	pending	pending
#*	JCB09768	*A. boulengeri*	18.378	-9.009	pending	pending	pending	pending
#*	JCB09788	*A. boulengeri*	18.406	-9.353	pending	pending	pending	pending
#*	JCB09802	*A. boulengeri*	18.593	-10.026	pending	pending	pending	pending

#*	JCB09808	*A. boueti*	18.527	-10.260	pending	pending	pending	pending
#*	JCB09854	*A. boulengeri*	18.357	-11.816	pending	pending	pending	pending
#*	JCB09907	*A. boueti*	17.618	-12.737	pending	pending	pending	pending
#*	JCB10617	*A. impalearis*	29.925	-9.627	pending	pending	pending	pending
#*	JCB10627	*A. impalearis*	28.518	-11.297	pending	pending	pending	pending
#*	JCB10700	*A. boueti*	16.409	-16.386	pending	pending	pending	pending
#*	JCB11032	*A. boueti*	16.288	-11.372	pending	pending	–	pending
#*	JCB11052	*A. boulengeri*	15.804	-11.305	pending	pending	pending	pending
#*	JCB11053	*A. boulengeri*	15.724	-11.270	pending	pending	pending	pending
#*	JCB11060	*A. boulengeri*	15.712	-11.265	pending	pending	pending	pending
#*	JCB11095	*A. boulengeri*	15.695	-11.238	pending	pending	pending	pending
#*	JCB11154	*A. boulengeri*	15.564	-10.982	pending	pending	pending	pending
#*	JCB11222	*A. boulengeri*	15.798	-10.805	pending	pending	pending	–
#*	JCB11241	*A. boueti*	15.837	-10.754	pending	pending	pending	pending
#*	JCB11247	*A. boulengeri*	15.948	-10.643	pending	pending	pending	pending
#*	JCB11252	*A. boulengeri*	16.055	-10.537	pending	pending	pending	pending
#*	JCB11259	*A. boulengeri*	16.038	-10.500	pending	pending	pending	pending
#*	JCB11281	*A. boulengeri*	16.060	-10.426	pending	pending	pending	pending
#*	JCB11291	*A. boulengeri*	16.195	-10.359	pending	pending	pending	pending
#*	JCB11298	*A. boulengeri*	16.544	-9.957	pending	pending	pending	pending
#*	JCB11315	*A. boueti*	16.645	-9.938	pending	pending	pending	pending
#*	JCB11323	*A. boulengeri*	16.665	-9.883	pending	pending	pending	pending
#*	JCB11337	*A. boulengeri*	16.550	-9.851	pending	pending	pending	pending
#*	JCB11341	*A. boulengeri*	16.420	-10.123	pending	pending	pending	pending
#*	JCB11349	*A. boulengeri*	16.490	-10.596	pending	pending	pending	pending
#*	JCB11363	*A. boulengeri*	16.927	-11.747	pending	pending	pending	pending
#*	JCB11364	*A. boulengeri*	16.942	-11.783	pending	pending	pending	pending
#*	JCB11366	*A. boulengeri*	16.965	-11.796	pending	pending	pending	pending
#*	JCB11367	*A. boulengeri*	16.973	-11.827	pending	pending	pending	pending
#*	JCB11370	*A. boulengeri*	17.019	-11.831	pending	pending	pending	pending
#*	JCB11373	*A. boulengeri*	16.932	-11.848	pending	pending	pending	pending
#*	JCB11439	*A. boueti*	22.249	-15.312	pending	pending	pending	pending
#*	MNCN-DNA09812	*A. boulengeri*	17.912	-12.128	pending	pending	pending	pending
#*	MNCN-DNA09831	*A. boulengeri*	17.893	-12.124	pending	pending	pending	pending
#*	MNCN-DNA09832	*A. boulengeri*	17.893	-12.124	pending	pending	pending	–
#*	MNCN-DNA09833	*A. boulengeri*	17.893	-12.124	pending	pending	pending	pending
#*	MNCN-DNA09840	*A. boulengeri*	18.550	-11.404	JN665054	pending	pending	–
#*	MNCN-DNA09841	*A. boulengeri*	18.550	-11.404	JN665052	pending	pending	pending
#*	MNCN-DNA09849	*A. boueti*	18.481	-10.946	pending	pending	pending	–
#*	MNCN-DNA09861	*A. boulengeri*	16.534	-10.798	pending	pending	pending	pending
#*	MNCN-DNA09862	*A. boulengeri*	16.534	-10.798	pending	pending	pending	pending
#*	MNCN-DNA09863	*A. boulengeri*	16.534	-10.798	pending	pending	pending	–
#*	MNCN-DNA09865	*A. boulengeri*	16.534	-10.798	pending	pending	pending	–
#*	MNCN-DNA20573	*A. agama*	15.528	-9.815	pending	pending	pending	–
#*	MNCN-DNA20577	*A. boulengeri*	16.624	-9.624	pending	pending	pending	–
#*	MNCN-DNA20580	*A. boueti*	16.624	-9.624	pending	pending	pending	pending
#*	MNCN-DNA20592	*A. boulengeri*	16.491	-10.830	pending	pending	pending	–
#*	MNCN-DNA20594	*A. boulengeri*	21.378	-12.976	pending	pending	pending	–

#*	MNCN-DNA20596	*A. boulengeri*	18.575	-9.806	pending	pending	pending	pending
#*	MNCN-DNA21485	*A. boulengeri*	20.505	-12.848	pending	pending	pending	pending
#*	MNCN-DNA21486	*A. boueti*	20.562	-12.547	pending	pending	pending	–
#*	MNCN-DNA21487	*A. agama*	14.520	-4.087	JN665051	pending	pending	pending
#*	MNCN-DNA21491	*A. boueti*	17.402	-12.352	pending	pending	pending	–
#*	MNCN-DNA39988	*A. spinosa*	24.564	34.903	pending	pending	pending	–
#*	MNCN-DNA39989	*A. spinosa*	24.562	34.924	pending	pending	pending	–
#*	MNCN-DNA39990	*A. spinosa*	22.193	36.393	pending	pending	pending	–
#	BEV02764	*A. tassiliensis*	19.001	9.335	JN665064	pending	–	–
	BEVT03092	*A. spinosa*	24.564	34.903	JN665065	–	–	–
	BEVT03093	*A. spinosa*	24.559	34.929	JN665066	–	–	–
	HDB00798	*A. impalearis*	29.580	-9.396	–	–	pending	–
#	JCB00104	*A. boulengeri*	17.943	-12.142	pending	–	pending	–
#	JCB01414	*A. boulengeri*	17.379	-12.342	pending	–	pending	–
#	JCB01640	*A. boulengeri*	20.519	-13.133	pending	–	pending	–
#	JCB01698	*A. boulengeri*	20.581	-13.136	pending	–	pending	–
#	JCB01863	*A. boulengeri*	19.863	-12.991	pending	–	pending	–
#	JCB01947	*A. boulengeri*	18.924	-11.839	pending	–	pending	–
#	JCB01973	*A. boulengeri*	18.755	-11.696	pending	–	–	–
#	JCB02047	*A. boulengeri*	17.635	-11.414	pending	–	pending	–
#	JCB02107	*A. boulengeri*	17.102	-10.956	pending	–	pending	–
#	JCB02128	*A. boulengeri*	16.980	-10.118	pending	–	pending	–
#	JCB02190	*A. boulengeri*	16.655	-9.708	pending	pending	pending	pending
#	JCB02288	*A. boulengeri*	16.688	-10.191	pending	pending	pending	pending
#	JCB02387	*A. boulengeri*	16.540	-10.801	pending	–	pending	–
#	JCB02600	*A. boulengeri*	17.101	-12.257	pending	–	pending	–
#	JCB02625	*A. boulengeri*	17.402	-12.389	pending	–	–	–
#	JCB02648	*A. boulengeri*	17.850	-12.186	pending	–	pending	–
#	JCB02874	*A. boueti*	20.835	-12.566	pending	–	pending	–
#	JCB02932	*A. boulengeri*	19.900	-13.347	pending	–	pending	–
#	JCB02974	*A. boulengeri*	18.643	-12.607	pending	–	pending	–
#	JCB02982	*A. boulengeri*	18.365	-12.307	pending	–	pending	–
	JCB03167	*A. boulengeri*	17.571	-12.179	–	–	pending	–
#	JCB03211	*A. boulengeri*	17.334	-12.078	–	pending	–	–
#	JCB03344	*A. boulengeri*	17.070	-12.208	pending	–	pending	–
#	JCB03430	*A. boulengeri*	17.371	-12.314	pending	–	pending	–
#	JCB03478	*A. boueti*	17.116	-13.575	pending	–	pending	–
#	JCB03480	*A. boueti*	17.082	-13.636	pending	pending	pending	pending
#	JCB04473	*A. boueti*	16.669	-16.422	pending	–	pending	–
#	JCB04503	*A. boueti*	16.378	-16.387	pending	pending	pending	pending
#	JCB04591	*A. boueti*	16.257	-13.658	pending	–	pending	–
#	JCB04623	*A. boulengeri*	15.989	-12.727	pending	–	pending	–
#	JCB04636	*A. boulengeri*	15.967	-12.678	pending	–	pending	pending
#	JCB04642	*A. boulengeri*	15.963	-12.681	pending	–	pending	–
#	JCB04762	*A. boulengeri*	15.682	-12.163	–	pending	pending	pending
#	JCB04795	*A. boulengeri*	15.957	-12.010	pending	–	pending	–
#	JCB04802	*A. boulengeri*	15.927	-11.969	pending	–	pending	–
#	JCB04806	*A. boulengeri*	15.901	-11.939	pending	–	pending	–

#	JCB04818	*A. boulengeri*	15.987	-11.940	pending	–	pending	–
#	JCB04880	*A. boulengeri*	17.152	-12.199	pending	–	pending	–
#	JCB04886	*A. boulengeri*	17.167	-12.173	pending	–	pending	–
#	JCB04889	*A. boulengeri*	17.168	-12.173	pending	–	pending	–
#	JCB04891	*A. boulengeri*	17.174	-12.172	pending	–	pending	–
#	JCB04892	*A. boulengeri*	17.180	-12.169	pending	–	pending	–
#	JCB04893	*A. boulengeri*	17.181	-12.168	pending	–	pending	–
#	JCB04896	*A. boulengeri*	17.172	-12.126	pending	–	pending	–
#	JCB04897	*A. boulengeri*	17.173	-12.139	pending	–	pending	–
#	JCB04898	*A. boulengeri*	17.162	-12.128	pending	–	pending	–
#	JCB04900	*A. boulengeri*	17.158	-12.116	pending	–	pending	–
#	JCB04905	*A. boulengeri*	17.158	-12.143	pending	–	pending	–
#	JCB04906	*A. boulengeri*	17.189	-12.160	pending	–	pending	–
#	JCB04911	*A. boulengeri*	17.196	-12.249	pending	–	pending	–
#	JCB04918	*A. boulengeri*	17.269	-12.284	pending	–	pending	pending
#	JCB04921	*A. boulengeri*	17.270	-12.245	pending	–	pending	pending
#	JCB05861	*A. boueti*	20.554	-12.692	pending	–	pending	pending
#	JCB06122	*A. boulengeri*	16.470	-12.485	pending	–	–	pending
#	JCB06252	*A. agama*	15.626	-13.147	pending	–	pending	pending
#	JCB06258	*A. agama*	15.742	-13.240	pending	–	pending	pending
	JCB06634	*A. paragama*	13.934	10.214	pending	pending	–	–
#	JCB06638	*A. boueti*	13.562	11.661	pending	–	–	–
#	JCB06771	*A. boueti*	14.748	10.293	pending	–	–	–
#	JCB06792	*A. agama*	13.617	4.031	pending	–	–	–
#	JCB07471	*A. agama*	15.448	-12.931	pending	–	–	–
#	JCB07789	*A. agama*	16.031	-11.619	pending	–	pending	pending
#	JCB07835	*A. boulengeri*	16.663	-11.345	pending	pending	pending	pending
#	JCB07838	*A. boulengeri*	16.685	-11.292	pending	pending	pending	pending
#	JCB07845	*A. boulengeri*	16.706	-11.208	pending	pending	pending	pending
#	JCB07852	*A. boulengeri*	16.729	-11.131	pending	pending	pending	pending
#	JCB07863	*A. boulengeri*	16.763	-11.223	pending	–	pending	pending
#	JCB07889	*A. boulengeri*	17.126	-12.093	pending	–	pending	pending
#	JCB07890	*A. boulengeri*	17.125	-12.094	pending	–	pending	pending
#	JCB07895	*A. boulengeri*	17.134	-12.090	pending	–	pending	pending
#	JCB07896	*A. boulengeri*	17.133	-12.090	pending	–	pending	pending
#	JCB07898	*A. boulengeri*	17.136	-12.090	pending	–	pending	pending
#	JCB09239	*A. boulengeri*	15.096	-12.244	pending	–	–	pending
#	JCB09329	*A. boulengeri*	16.378	-11.081	pending	pending	pending	pending
#	JCB09470	*A. boulengeri*	14.388	-11.386	pending	pending	pending	pending
#	JCB09797	*A. boulengeri*	18.579	-9.818	pending	pending	–	–
#	JCB09822	*A. boulengeri*	18.511	-11.013	pending	–	–	–
#	JCB09826	*A. boulengeri*	18.563	-11.189	pending	pending	–	–
#	JCB09832	*A. boulengeri*	18.443	-11.387	pending	–	–	–
#	JCB09883	*A. boulengeri*	18.206	-11.731	pending	pending	–	–
#	JCB09890	*A. boulengeri*	17.982	-11.945	pending	–	–	–
#	JCB11246	*A. boulengeri*	15.948	-10.643	–	pending	pending	pending
#	JCB11266	*A. boulengeri*	16.051	-10.539	pending	–	pending	–
	MNCN-DNA20574	*A. agama*	15.528	-9.815	–	–	pending	–

	MNCN-DNA20576	*A. agama*	15.430	-11.926	–	–	pending	–
#	MNCN-DNA20579	*A. boueti*	16.624	-9.624	JN665053	–	pending	–
#	MNCN-DNA20585	*A. boulengeri*	20.581	-13.127	pending	–	pending	–
#	MNCN-DNA20586	*A. boulengeri*	20.581	-13.127	pending	pending	–	–
	MNCN-DNA20588	*A. boulengeri*	16.491	-10.830	–	–	pending	–
	MNCN-DNA20589	*A. boulengeri*	16.491	-10.830	–	–	pending	–
	MNCN-DNA20590	*A. boulengeri*	16.491	-10.830	–	–	pending	–
	MNCN-DNA20591	*A. boulengeri*	16.491	-10.830	–	–	pending	–
	MNCN-DNA20593	*A. boulengeri*	16.491	-10.830	–	–	pending	–
#	MNCN-DNA21483	*A. boulengeri*	20.573	-13.099	pending	pending	–	–
#	MNCN-DNA21484	*A. boulengeri*	20.930	-11.621	pending	pending	–	–
#	MNCN-DNA21492	*A. boulengeri*	19.083	-12.350	pending	–	pending	–
#	MNCN-DNA09842	*A. boulengeri*	18.550	-11.404	–	pending	pending	–
#	MNCN-DNA09845	*A. boulengeri*	18.481	-10.946	pending	pending	–	–
#	MNCN-DNA09864	*A. boulengeri*	16.534	-10.798	–	pending	pending	–
#	MNCN-DNA09866	*A. boulengeri*	16.534	-10.798	–	pending	–	–
#	MNCN-DNA09867	*A. boulengeri*	16.534	-10.798	–	pending	pending	–
G#	7005X	*A. agama*			JF520660	–	–	–
G#	8540X	*A. agama*			JN791275	–	–	–
G#	8623X	*A. agama*			JN791274	–	–	–
G#	TR2303	*A. agama*			JF520694	–	–	–
G#	TR2304	*A. agama*			JF520695	–	–	–
G#	TR2374	*A. agama*			JF520665	–	–	–
G#	TR2375	*A. agama*			JF520666	–	–	–
G#	TR2381	*A. agama*			JF520667	–	–	–
G#	TR2384	*A. agama*			JF520668	–	–	–
G#	TR2394	*A. agama*			JF520669	–	–	–
G#	TR2956	*A. agama*			JF520664	–	–	–
G#	TR3019	*A. agama*			JF520661	–	–	–
G#	TR3109	*A. agama*			JF520663	–	–	–
G#	TR3466	*A. agama*			JF520681	–	–	–
G#	TR3470	*A. agama*			JF520682	–	–	–
G#	6487X	*A. agama*			JF520683	–	–	–
G#	MCZ.184561	*A. agama*			GU128433	GU128470	–	–
G#	MHNG.2689.53	*A. agama*			GU133310	–	–	–
G#	MVZ.238891	*A. agama*			GU128443	GU128480	–	–
G#	MVZ.249617	*A. agama*			GU128439	GU128476	–	–
G#	TR1730	*A. agama*			JF520693	–	–	–
G#	TR3199	*A. agama*			JF520659	–	–	–
G#	TR3209	*A. agama*			JF520657	–	–	–
G#	TR3238	*A. agama*			JF520658	–	–	–
G#	TR3306	*A. agama*			JF520656	–	–	–
G#	ZFMK.73185	*A. agama*			GU128436	GU128473	–	–
G#	NCSM.76789	*A. agama*			JX668203	–	–	–
G#	LSUMZ.H20336	*A. agama*			GU128437	GU128474	–	–
G#	TR2644	*A. agama*			JF520678	–	–	–
G#	TR2645	*A. agama*			JF520679	–	–	–
G#	TR3112	*A. agama*			JF520662	–	–	–

G#	ZMB.71577	*A. agama*	JX668205	JX857543	–	–
G#	ZFMK.73846	*A. agama*	GU133312	–	–	–
G#	TR2535	*A. agama*	JF520670	–	–	–
G#	TR2537	*A. agama*	JF520671	–	–	–
G#	TR2538	*A. agama*	JF520672	–	–	–
G#	TR2886	*A. agama*	JF520690	–	–	–
G#	TR3558	*A. agama*	JF520673	–	–	–
G#	TR3559	*A. agama*	JF520674	–	–	–
G#	TR3807	*A. agama*	JF520691	–	–	–
G#	TR3820	*A. agama*	JF520675	–	–	–
G#	TR3862	*A. agama*	JF520692	–	–	–
G#	TR3891	*A. agama*	JF520676	–	–	–
G#	TR3939	*A. agama*	JF520677	–	–	–
G#	TR2851	*A. agama*	JF520684	–	–	–
G#	TR3504	*A. agama*	JF520688	–	–	–
G#	TR3505	*A. agama*	JF520689	–	–	–
G#	TR3575	*A. agama*	JF520686	–	–	–
G#	TR3590	*A. agama*	JF520687	–	–	–
G#	2901I	*A. agama*	JX668202	JX857626	–	–
G#	AMNH.109799	*A. agama*	GU128441	GU128478	–	–
G#	MNCN.20575	*A. agama*	JN665050	–	–	–
G#	TR1893	*A. agama*	JF520680	–	–	–
G#	ZFMK.76838	*A. agama*	GU128442	GU128479	–	–
G#	MVZ.235766	*A. boueti*	GU128454	GU128491	–	–
G#	MNCN41776	*A. boueti*	AY522926	–	–	–
G#	MNCN41777	*A. boueti*	AY522927	–	–	–
G#	MNCN41778	*A. boueti*	AY522928	–	–	–
G#	MNCN41779	*A. boueti*	AY522929	–	–	–
G#	TR107	*A. boueti*	JF520716	–	–	–
G#	6015X	*A. boueti*	JF520714	–	–	–
G#	6020X	*A. boueti*	JF520705	–	–	–
G#	6069X	*A. boueti*	JF520712	–	–	–
G#	6075X	*A. boueti*	JF520715	–	–	–
G#	6088X	*A. boueti*	JF520708	–	–	–
G#	6089X	*A. boueti*	JF520709	–	–	–
G#	6092X	*A. boueti*	JF520713	–	–	–
G#	6132X	*A. boueti*	JF520707	–	–	–
G#	6251X	*A. boueti*	JX668158	–	–	–
G#	6253X	*A. boueti*	JX668159	JX857557	–	–
G#	6309X	*A. boueti*	JF520710	–	–	–
G#	7297X	*A. boueti*	JF520711	–	–	–
G#	MNHN.IV	*A. boueti*	JX668161	JX857551	–	–
G#	MVZ.238892	*A. boueti*	JX668162	JX857613	–	–
G#	TR3508	*A. boueti*	JF520706	–	–	–
G#	ZFMK.80057	*A. boueti*	GU133313	–	–	–
G#	MNCN.20582	*A. boulengeri*	JN665055	–	–	–
G#	ZFMK.76868	*A. boulengeri*	JX668164	–	–	–
G#	MNCN.20584	*A. boulengeri*	JN665056	–	–	–

G#	MNCN.20595	*A. boulengeri*	JN665057	–	–	–
G#	MNCN.20597	*A. boulengeri*	JN665059	–	–	–
G#	MNCN.20598	*A. boulengeri*	JN665058	–	–	–
G#	MNHN.I	*A. boulengeri*	GU133324	JX857584	–	–
G#	MVZ.235764	*A. boulengeri*	GU128449	GU128486	–	–
G#	haplotype.12	*A. impalearis*	AJ414677	–	–	–
G#	haplotype.15	*A. impalearis*	AJ414678	–	–	–
G#	haplotype.27	*A. impalearis*	AJ414684	–	–	–
G#	haplotype.31	*A. impalearis*	AJ414686	–	–	–
G#	haplotype.33	*A. impalearis*	AJ414687	–	–	–
G#	haplotype.34	*A. impalearis*	AJ414688	–	–	–
G#	haplotype.35	*A. impalearis*	AJ414689	–	–	–
G#	haplotype.1	*A. impalearis*	AJ414672	–	–	–
G#	haplotype.18	*A. impalearis*	AJ414679	–	–	–
G#	haplotype.21	*A. impalearis*	AJ414680	–	–	–
G#	haplotype.22	*A. impalearis*	AJ414681	–	–	–
G#	haplotype.23	*A. impalearis*	AJ414682	–	–	–
G#	haplotype.25	*A. impalearis*	AJ414683	–	–	–
G#	haplotype.28	*A. impalearis*	AJ414685	–	–	–
G#	haplotype.6	*A. impalearis*	AJ414673	–	–	–
G#	haplotype.7	*A. impalearis*	AJ414674	–	–	–
G#	haplotype.8	*A. impalearis*	AJ414675	–	–	–
G#	haplotype.9	*A. impalearis*	AJ414676	–	–	–
G#	TR1838	*A. impalearis*	JF520722	–	–	–
G#	6026X	*A. tassiliensis*	JF520747	–	–	–
G#	6070X	*A. tassiliensis*	JF520750	–	–	–
G#	6080X	*A. tassiliensis*	JF520749	–	–	–
G#	6090X	*A. tassiliensis*	JF520748	–	–	–
G#	MNHN	*A. tassiliensis*	JN665063	–	–	–
G#	BEVT439	*A. tassiliensis*	JX128166	JX128198	–	–
G#	BEVT441	*A. tassiliensis*	JX128167	JX128199	–	–

* Samples used to construct the tree in Fig. B1.

Samples used to calculate the summary genetic diversity statistics in Table 5.3.

G Samples exclusively from GenBank.

Table B. 2. Best partition scheme according to PartitionFinder. N = number of included sequences; Length = aligned base-pair fragment length; V = number of variable positions; Pi = number of parsimony informative sites.

Initial partition	Subset Partitions	Best Model	N, Length, V, Pi
By gene	ND4	TVM+I+G*	ND4: 341, 699, 502, 448
	16S, tRNAs	GTR+I+G	t-RNAs: 327, 196, 141, 119
	c-mos, NT3	K81+I+G	16S: 341, 522, 267, 212
By codon	ND4_1, tRNAs	GTR+I+G	c-mos: 335, 570, 128, 76
	CMOS_3, ND4_2	TVM+I+G	NT3: 301, 669, 224, 157
	ND4_3	TIM+G	
	16S	GTR+I+G	
	CMOS_1, CMOS_2, NT3_1, NT3_2	K81+I+G	
	NT3_3	K80+G	

* for the alignment used in the time-calibrated phylogeny, the best model was GTR+I+G.

Table B. 3. Summary genetic diversity and demographic statistics for the nuclear markers, based on the 341 samples used to construct the phylogenetic tree in Fig. B1. L = minimum length, excluding sites with gaps and missing data; P = number of polymorphic sites, excluding sites with gaps and missing data; N = number of phased haplotypes; *h* = number of unique haplotypes; *Hd* = Haplotype diversity; π = nucleotide diversity; R2 = Ramos-Onsins and Rozas R2 statistic; *D* = Tajima's *D* (significant values in bold); F_S = Fu's F_S statistic.

	c-mos									NT3								
Group	**L**	**P**	**N**	***h***	***Hd***	**π**	**R2**	**D**	***FS***	**L**	**P**	**N**	***h***	***Hd***	**π**	**R2**	**D**	***FS***
A. boueti	570	12	124	12	0.595+-0.024	0.00131+-0.00012	0.0347	-1.71649	-8.07	657	37	120	48	0.909+-0.016	0.00546+-0.00033	0.0463	-1.50561	-44.62
boueti C	570	7	72	8	0.597+-0.038	0.00137+-0.00017	0.0578	-1.14033	-3.521	657	26	68	33	0.936+-0.016	0.00341+-0.00028	0.0389	**-1.90123**	-36.541
boueti E	570	4	24	4	0.239+-0.113	0.00058+-0.00031	0.1179	**-1.88381**	-2.331	657	14	26	11	0.674+-0.104	0.00238+-0.00061	0.0513	**-1.9719**	-6.26
boueti W	570	1	28	2	0.071+-0.065	0.00013+-0.00011	0.1856	-1.15142	-1.155	657	11	26	5	0.289+-0.115	0.00150+-0.00082	0.0951	**-2.18466**	-0.824
A. boulengeri	570	11	320	12	0.685+-0.018	0.00163+-0.00008	0.0429	-1.05206	-4.67	654	29	294	37	0.823+-0.014	0.0023+-0.00010	0.0254	**-1.83889**	-37.276
boulengeri E	570	1	84	2	0.07+-0.038	0.00012+-0.00007	0.0349	-0.77432	-0.887	654	3	76	4	0.127+-0.052	0.00020+-0.00008	0.0549	-1.49016	-3.823
boulengeri N	570	5	122	5	0.571+-0.022	0.00117+-0.00009	0.0727	-0.58863	-0.54	654	15	106	19	0.821+-0.021	0.00257+-0.00014	0.054	-1.13318	-10.719
boulengeri S	570	6	108	8	0.58+-0.049	0.00136+-0.00015	0.0652	-0.70837	-2.993	654	17	106	20	0.661+-0.050	0.00146+-0.00017	0.0283	**-1.97879**	-20.055
A. impalearis	570	3	14	4	0.571+-0.132	0.00135+-0.00043	0.1352	-0.56505	-0.99	657	17	14	12	0.978+-0.035	0.00664+-0.00079	0.0999	-0.75857	-6.273
A. tassiliensis	570	5	26	5	0.622+-0.061	0.00158+-0.00035	0.1078	-0.89067	-1.07	657	13	20	6	0.684+-0.684	0.00546+-0.00169	0.1344	-0.07929	1.471

Table B. 4. Individual model evaluation scores and variable contribution to models. TSS = average (and standard deviation) TSS scores; N = number of replicates with TSS > 0.75; for each modelling technique. Replicates with TSS < 0.75 were not used for projections or ensemble forecasting. Consensus values comprise all techniques. Variables with an average contribution >0.5 are signalled in bold. For variables names see Table 5.4.

Sp.	Technique	TSS	N	bio_1	bio_2	bio_5	bio_6	bio_7	bio_12	bio_14
A. boueti	ANN	0.815 (0.04)	74	**0.648 (0.044)**	0.071 (0.031)	**0.902 (0.025)**	**0.631 (0.015)**	**1 (0)**	0.396 (0.066)	0.036 (0.033)
	GAM	0.852 (0.047)	91	**0.641 (0.08)**	0.402 (0.062)	**0.858 (0.116)**	**0.902 (0.19)**	**0.743 (0.124)**	**0.57 (0.068)**	0.053 (0.035)
	GLM	0.85 (0.035)	96	0.456 (0.18)	0.313 (0.09)	0.317 (0.164)	**0.688 (0.167)**	**0.731 (0.143)**	**0.559 (0.152)**	0.023 (0.029)
	MAXENT	0.821 (0.036)	75	0.357 (0.104)	0.123 (0.102)	0.443 (0.084)	0.418 (0.128)	**0.557 (0.122)**	**0.85 (0.057)**	0.012 (0.019)
	Consensus			**0.525 (0.168)**	0.227 (0.155)	**0.629 (0.277)**	**0.659 (0.223)**	**0.758 (0.194)**	**0.594 (0.188)**	0.031 (0.033)
A. boulengeri	ANN	0.882 (0.043)	98	0.329 (0.06)	0.5 (0.044)	**0.966 (0.029)**	**0.815 (0.163)**	**0.9 (0.118)**	0.244 (0.11)	0.058 (0.029)
	GAM	0.937 (0.023)	100	**0.504 (0.087)**	**0.604 (0.025)**	**0.837 (0.086)**	**0.897 (0.145)**	**0.843 (0.106)**	0.277 (0.068)	0.022 (0.017)
	GLM	0.913 (0.026)	99	**0.666 (0.177)**	0.452 (0.112)	**0.509 (0.154)**	**0.819 (0.135)**	**0.742 (0.111)**	0.473 (0.145)	0.027 (0.03)
	MAXENT	0.802 (0.037)	35	**0.67 (0.082)**	**0.803 (0.018)**	**0.532 (0.109)**	0.237 (0.094)	**0.607 (0.072)**	0.307 (0.057)	0.005 (0.008)
	Consensus			**0.542 (0.179)**	**0.59 (0.149)**	**0.712 (0.222)**	**0.692 (0.298)**	**0.773 (0.152)**	0.325 (0.134)	0.028 (0.03)
A. impalearis	ANN	0.918 (0.054)	95	**0.954 (0.106)**	0.31 (0.019)	**0.793 (0.191)**	**0.663 (0.216)**	**0.665 (0.291)**	0.477 (0.042)	0.161 (0.024)
	GAM	0.882 (0.06)	73	**0.794 (0.123)**	**0.558 (0.04)**	**0.779 (0.17)**	**0.799 (0.131)**	**0.775 (0.118)**	**0.541 (0.063)**	0.381 (0.034)
	GLM	0.943 (0.033)	100	**0.725 (0.266)**	0.381 (0.158)	**0.534 (0.248)**	0.443 (0.267)	0.329 (0.171)	**0.649 (0.23)**	0.249 (0.13)
	MAXENT	0.874 (0.046)	98	**0.729 (0.073)**	0.456 (0.159)	0.457 (0.194)	0.252 (0.139)	0.3 (0.175)	**0.696 (0.186)**	0.131 (0.085)
	Consensus			**0.801 (0.187)**	0.42 (0.146)	**0.634 (0.252)**	**0.526 (0.286)**	**0.505 (0.286)**	**0.593 (0.178)**	0.223 (0.124)
A. tassiliensis	ANN	0.889 (0.066)	93	**0.709 (0.301)**	0.42 (0.214)	**0.764 (0.327)**	**0.798 (0.347)**	**0.797 (0.337)**	0.5 (0.216)	0.416 (0.247)
	GAM	0.853 (0.076)	68	**0.86 (0.032)**	**0.559 (0.103)**	**0.954 (0.076)**	**0.934 (0.108)**	**0.97 (0.041)**	**0.675 (0.128)**	0.2 (0.178)
	GLM	0.92 (0.05)	84	**0.904 (0.042)**	**0.582 (0.172)**	**0.966 (0.05)**	**0.957 (0.045)**	**0.511 (0.143)**	0.417 (0.15)	0.118 (0.117)
	MAXENT	0.87 (0.07)	39	**0.831 (0.036)**	0.288 (0.234)	**0.871 (0.051)**	**0.865 (0.082)**	**0.779 (0.163)**	**0.79 (0.081)**	**0.545 (0.289)**
	Consensus			**0.824 (0.172)**	0.458 (0.223)	**0.886 (0.192)**	**0.886 (0.2)**	**0.762 (0.26)**	**0.596 (0.211)**	0.325 (0.277)

Table B. 5. Niche comparisons at lineage, species, and intra-genus branch levels, testing for similarity (above, grey), and dissimilarity (below). Significant (p<0.05) similarity tests are marked in grey. Equivalency tests are not directly comparable with those calculated using the old methodology (Broennimann et al., 2012; see discussion and Text B2).

Variables as models				
Comparison	D	Equivalency	Similarity_BA	Similarity_AB
boue_C -> boue_E	0.16	0.97	0.08	0.09
boue_C -> boue_W	0.11	1.00	0.08	0.07
boue_E -> boue_W	0.06	1.00	0.24	0.23
boul_E -> boul_N	0.13	1.00	0.05	0.03
boul_E -> boul_S	0.58	0.00	0.00	0.00
boul_N -> boul_S	0.21	0.00	0.01	0.01
boue -> boul	0.04	1.00	0.37	0.31
boue -> imp	0.04	1.00	0.42	0.37
boue -> tass	0.03	1.00	0.57	0.56
boul -> imp	0.00	1.00	1.00	1.00
boul -> tass	0.03	1.00	0.23	0.21
imp -> tass	0.06	1.00	0.31	0.36
bit -> boul	0.01	1.00	0.07	0.06
boue_C -> boue_E	0.16	0.04	0.89	0.88
boue_C -> boue_W	0.11	0.01	0.93	0.93
boue_E -> boue_W	0.06	0.00	0.74	0.77
boul_E -> boul_N	0.13	0.00	0.97	0.98
boul_E -> boul_S	0.58	1.00	1.00	1.00
boul_N -> boul_S	0.21	1.00	0.99	1.00
boue -> boul	0.04	0.00	0.63	0.62
boue -> imp	0.04	0.00	0.59	0.63
boue -> tass	0.03	0.00	0.40	0.43
boul -> imp	0.00	0.00	0.72	0.71
boul -> tass	0.03	0.00	0.77	0.72
imp -> tass	0.06	0.00	0.67	0.69
bit -> boul	0.01	0.00	0.93	0.92
All Bioclim	(B)			
Comparison	D	Equivalency	Similarity_BA	Similarity_AB
boue_C -> boue_E	0.15	0.72	0.12	0.08
boue_C -> boue_W	0.11	0.95	0.13	0.11
boue_E -> boue_W	0.03	1.00	0.29	0.34
boul_E -> boul_N	0.15	1.00	0.06	0.05
boul_E -> boul_S	0.60	0.02	0.03	0.02
boul_N -> boul_S	0.24	0.36	0.04	0.03
boue -> boul	0.17	1.00	0.06	0.08
boue -> imp	0.04	1.00	0.41	0.38
boue -> tass	0.03	1.00	0.59	0.57
boul -> imp	0.05	1.00	0.37	0.30
boul -> tass	0.04	1.00	0.35	0.34
imp -> tass	0.05	1.00	0.41	0.41
bit -> boul	0.01	1.00	0.21	0.18
boue_C -> boue_E	0.15	0.26	0.93	0.93
boue_C -> boue_W	0.11	0.05	0.89	0.91

boue_E -> boue_W	0.03	0.00	0.70	0.69
boul_E -> boul_N	0.15	0.00	0.94	0.92
boul_E -> boul_S	0.60	0.97	0.98	0.98
boul_N -> boul_S	0.24	0.67	0.98	0.96
boue -> boul	0.17	0.00	0.93	0.90
boue -> imp	0.04	0.00	0.59	0.59
boue -> tass	0.03	0.00	0.45	0.40
boul -> imp	0.05	0.00	0.74	0.68
boul -> tass	0.04	0.00	0.68	0.65
imp -> tass	0.05	0.00	0.55	0.58
bit -> boul	0.01	0.00	0.81	0.84
Bioclim + Envirem	(B)			
Comparison	D	Equivalency	Similarity_BA	Similarity_AB
boue_C -> boue_E	0.13	0.90	0.05	0.04
boue_C -> boue_W	0.13	0.98	0.14	0.11
boue_E -> boue_W	0.06	1.00	0.25	0.28
boul_E -> boul_N	0.16	1.00	0.05	0.03
boul_E -> boul_S	0.61	0.03	0.02	0.01
boul_N -> boul_S	0.23	0.61	0.04	0.03
boue -> boul	0.18	1.00	0.08	0.08
boue -> imp	0.03	1.00	0.52	0.52
boue -> tass	0.02	1.00	0.62	0.63
boul -> imp	0.03	1.00	0.38	0.36
boul -> tass	0.03	1.00	0.42	0.43
imp -> tass	0.06	1.00	0.43	0.41
bit -> boul	0.00	1.00	0.19	0.20
boue_C -> boue_E	0.13	0.14	0.94	0.93
boue_C -> boue_W	0.13	0.01	0.83	0.86
boue_E -> boue_W	0.06	0.00	0.73	0.72
boul_E -> boul_N	0.16	0.00	0.97	0.98
boul_E -> boul_S	0.61	0.98	0.99	0.98
boul_N -> boul_S	0.23	0.42	0.97	0.97
boue -> boul	0.18	0.00	0.93	0.93
boue -> imp	0.03	0.00	0.54	0.49
boue -> tass	0.02	0.00	0.41	0.40
boul -> imp	0.03	0.00	0.63	0.65
boul -> tass	0.03	0.00	0.53	0.56
imp -> tass	0.06	0.00	0.55	0.64
bit -> boul	0.00	0.00	0.82	0.83

Table B. 6. Genetic uncorrected *p*-distances and summary diversity statistics for the individual mitochondrial markers including all sequences available in GenBank (Table B1). Values pertaining to 16S are in bold font. N = number of samples; *h* = number of haplotypes; *Hd* = haplotype diversity; π = nucleotide diversity. For diversity values corresponding to the samples used to build the phylogenetic tree in Fig. B1, see Table B3.

ND4+tRNAs	A. agama	A. boueti	A. boulengeri	A. impalearis	A. tassiliensis	N	*h*	*Hd*	π
16s									
A. agama	2.7+-0.3	22.4+-1.2	22.9+-1.2	21.5+-1.2	22.9+-1.3	54	34	0.945+-0.023	0.02617+-0.00397
	1.3+-0.3					**105**	**31**	**0.913+-0.015**	**0.01412+-0.00056**
A. boueti		2.7+-0.3	24.2+-1.2	8.8+-0.8	15.1+-1	70	41	0.971+-0.010	0.02785+-0.00167
	10.8+-1.3	**1.2+-0.3**				**96**	**31**	**0.94+-0.011**	**0.01059+-0.00037**
A. boulengeri			6.9+-0.5	23.5+-1.2	22.9+-1.2	189	87	0.969+-0.006	0.07312+-0.00195
	13.2+-1.3	**13.5+-1.4**	**3.7+-0.5**			**243**	**30**	**0.876+-0.011**	**0.03426+-0.00093**
A. impalearis				2.2+-0.3	14.7+-1	8	6	0.929+-0.084	0.02106+-0.00470
	10.3+-1.3	**4.5+-0.8**	**13.3+-1.4**	**1.1+-0.3**		**27**	**18**	**0.915+-0.047**	**0.01122+-0.00112**
A. tassiliensis					3.5+-0.4	16	8	0.808+-0.093	0.03894+-0.00615
	10.8+-1.3	**8.4+-1.1**	**13.7+-1.4**	**7.9+-1.1**	**1.8+-0.3**	**21**	**5**	**0.767+-0.067**	**0.0158+-0.00223**

Appendix C – Supplementary material of chapter 6.1

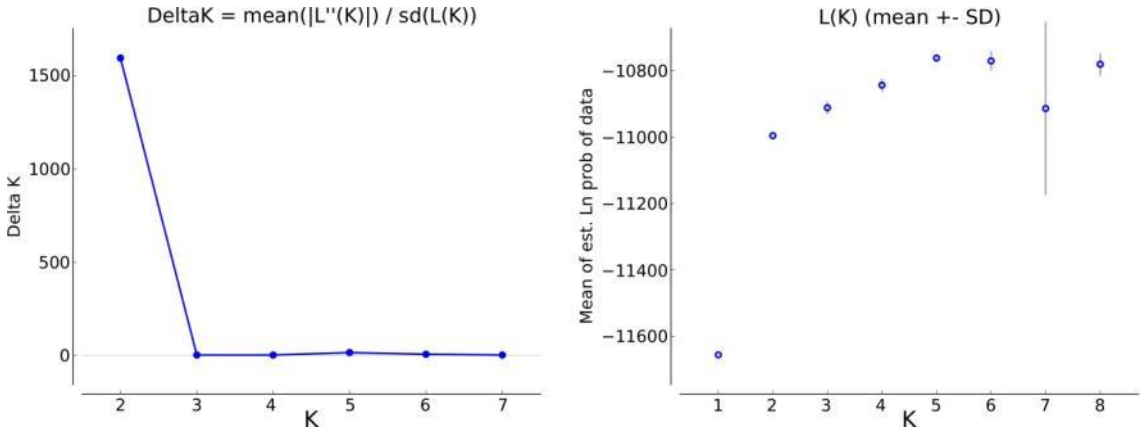

Figure C. 1. STRUCTURE HARVESTER plots used to determine optimal K. Left: delta K values, following Evanno et al. (2005); right: mean Ln probability of each K value.

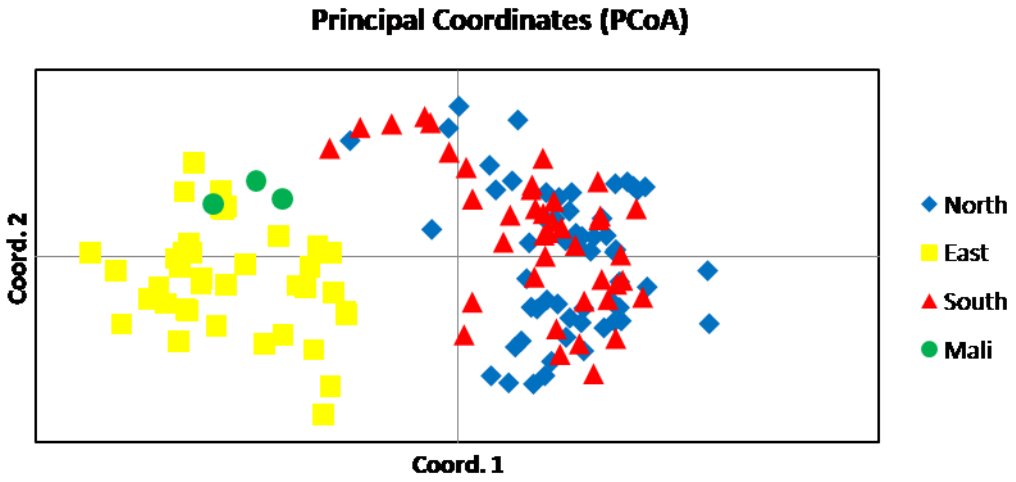

Figure C. 2. Principal coordinates analysis (PCoA) representing the genetic distance among samples of *Agama boulengeri*, based on 17 microsatellite loci. Samples are coloured according to the respective mitochondrial lineage.

Table C. 1. Summary statistics of genetic diversity and demography for *Agama boulengeri* and East (E), North (N) and South (S) mitochondrial lineages. Values correspond to the sum (L, P) or average (remaining statistics) of the values for mitochondrial (mt) and nuclear (nuc) markers, obtained from Gonçalves et al. (in prep.). L = length, excluding sites with gaps; P = number of polymorphic sites, excluding sites with gaps; N = number of samples or phased sequences; *h* = number of unique haplotypes; *Hd* = Haplotype diversity; π = nucleotide diversity; R2 = Ramos-Onsins and Rozas R2 statistic; *D* = Tajima's *D* (significant values in bold font); F_S = Fu's F_S statistic.

mt	L	P	N	h	Hd	π	R2	D	Fs
All	1155	255	165	19	0.937	0.057595	0.14165	1.583065	0.608
E	1195	48	46	14.5	0.716	0.007355	0.1077	-0.1459	-1.9085
N	1231	54	62	23	0.824	0.00563	0.0638	-1.10952	-10.9435
S	1239	89	54	19.5	0.8405	0.007305	0.0578	-1.47062	-5.3785
nuc									
All	1224	40	307	24.5	0.754	0.001965	0.03415	-1.44548	-20.974
E	1224	4	80	3	0.0985	0.00016	0.0449	-1.13224	-2.355
N	1224	20	114	12	0.696	0.00187	0.06335	-0.86091	-5.6295
S	1224	23	107	14	0.6205	0.00141	0.04675	-1.34358	-11.524

Appendix D – Other publications

D.1. Nuclear and mitochondrial markers reveal the existence of several geographically concordant lineages within a Sahelian gecko species, *Ptyodactylus ragazzii*

Amphibia-Reptilia 34 (2013): 85-93

Nuclear and mitochondrial markers reveal the existence of several geographically concordant lineages within a Sahelian gecko species, *Ptyodactylus ragazzii*

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Abstract. The genetic diversity within *Ptyodactylus ragazzii* was analysed for the first time across the Western part of its range. We have used two mitochondrial (12s rRNA and 16s rRNA) and one nuclear (Cmos) marker to compare results directly with other related *Ptyodactylus* species, *P. oudrii* and *P. hasselquistii*. Results show high levels of intraspecific variability, with at least three divergent mtDNA lineages that have different haplotypes for Cmos and that are geographically concordant. *P. ragazzii* from Mauritania is probably a distinct species and possibly other lineages too, such as those from the Aïr Mountains in Niger, although more nuclear markers are needed to confirm this. All analysed *Ptyodactylus* species appear to be cryptic species complexes containing multiple deeply divergent forms, highlighting the need for a careful reassessment of the taxonomy of the whole genus.

Keywords: 12s rRNA, 16s rRNA, Cmos, genetic variability, phylogeny, *Ptyodactylus*.

Introduction

The Western Sahel, covering Mauritania, Southern Algeria, Mali and Niger, has been identified as a biogeographic crossroad where the southern range limit of species with Palearctic affinity meets with the northern range limit of species with Afro-tropical affinity (Dekeyser and Villiers, 1956; Padial et al., 2010). However, this contact of Afro-tropical and Palearctic taxa is not restricted to the Sahel. Mountains in the Sahara, including the Aïr and Hoggar mountains, are known to act like islands surrounded by the desert, and constitute refugia for isolated populations of fishes, amphibians and reptiles (Froufe, Brito and Harris, 2009; Trape, 2009; Brito et al., 2011). The presence of endemisms and relict populations in the mountains can be related to the Saharan historical range oscillations in the last few million years, which have largely affected the range and composition of North African biodiversity (Le Houérou, 1997).

By contrast with Southern Europe and the Mediterranean biotypes of North Africa, where numerous studies have been carried out on herpetofauna phylogeographic patterns (e.g. Pinho, Harris and Ferrand, 2007; Rato and Harris, 2008; Perera et al., 2012) few are available for the more desertic regions of North Africa (e.g. Rato et al., 2007; Froufe, Brito and Harris, 2009; Gonçalves et al., 2012). Interestingly, the genetic studies that sampled extensively within widespread herpetofauna species from North Africa often reveal geographically structured phylogenetic patterns suggesting the existence of morphologically cryptic taxa (e.g. Zangari, Cimmaruta and Nascetti, 2006; Recuero et al., 2007). This is particularly true for geckos from the region; of studies from Morocco for example, all species analysed have included deeply divergent mtDNA lineages, at a level that could be considered cryptic species (Perera and Harris, 2010; Barata et al., 2012; Rato, Carranza and Harris, 2012).

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Ptyodactylus (Reptilia: Sauria: Gekkonidae) is one of the most distinctive genera of geckos, easily recognizable by their widely dilated toes formed by two divergent series of lamellae, similar to a fan (Schleich, Kästle and Kabisch, 1996). Six species have been described on the basis of their morphology and distribution (Sindaco and Jeremcenko, 2008), but relatively high morphological variation at both intra and inter specific levels particularly in the Middle East, means that different populations are sometimes assigned to different species depending on the taxonomist (Arnold, 1986; Werner and Sivan, 1993, 1994). Only one detailed study provided insights into the molecular variability within a singles species. Perera and Harris (2010) examined the genetic variability of *P. oudrii* across the Maghreb using two mitochondrial (12s rRNA and 16s rRNA) and two nuclear (Cmos and ACM4) markers. These authors found high levels of intraspecific variability, indicative of a species complex.

P. ragazzii is a species found from the central Sahara mountains (Adrar des Iforas, Aïr, Tassili, Hoggar, Tibesti) and the sahelo-soudanian areas of Mauritania, to Sudan and Somalia. It inhabits rocky cliffs and accumulations of large boulders, but can also be found in man-made walls in human settlements (Trape et al., 2012). With the exception of three individuals from Egypt, Mali and Algeria, sequenced for only mtDNA genes (Perera and Harris, 2010) no study to date evaluated the levels of genetic diversity within *P. ragazzii*.

The present study aims to depict the overall genetic pattern of *P. ragazzii* in western-central Africa, using both mitochondrial and nuclear DNA sequences. Additionally, *P. ragazzii* has recently been reported from a single locality in Mauritania (De la Riva and Padial, 2008), and therefore we also included samples from this region. We tested the specific predictions that: (1) high genetic diversity is expected, as found both in *P. oudrii* and in all other geckos from Northwest Africa; (2) divergent lineages will be geographically structured as also found in *P. oudrii*.

Materials and methods

DNA extraction, amplification and sequencing

A total of 26 samples of *P. ragazzii* from 16 localities covering the NW range of the species were analysed (table 1, fig. 1). In order to compare *P. ragazzii* relative to other *Ptyodactylus* species, we also analysed three new individuals of *P. oudrii* from Algeria (table 1) and included mtDNA sequences from 29 individuals of *P. oudrii*, two of *P. hasselquistii* and three from *P. ragazzii* (Perera and Harris, 2010), plus two nDNA Cmos sequences of *P. hasselquistii* and *P. guttatus* available in GenBank (Accession Numbers EU293681 and EU293682; Gamble et al., 2008). Mitochondrial sequences of *Phyllodactylus xanti* (Accession Number FJ662571, Blair et al., 2009) and *Tarentola mauritanica* (Accession Numbers AY828480 and AY828456, Harris et al., 2004) were used as outgroups.

Total genomic DNA was extracted from small pieces of tail using standard saline methods (Sambrook, Fritsch and Maniatis, 1989). Fragments of two mitochondrial regions (12s rRNA and 16s rRNA) and one nuclear region (Cmos) were analysed to compare results directly with Perera and Harris, 2010. Primers used in both amplification and sequencing were 12sa and 12sb for the 12s rRNA (Kocher et al., 1989), 16sa and 16sb for the 16s rRNA (Hedges, Bezy and Maxson, 1991), and G73 and G74 for the Cmos (Saint et al., 1998). Additionally we designed a new set of 12s rRNA primers (PTY12sL: ATTAGATACCCCACTATGCCCAGCCATTA; PTY12sH: GGTGACGGGCGGTGTGTGCG) and used them for some samples. PCR mixes were carried out in a 25 μl total volume, following conditions described in Harris, Arnold, and Thomas (1998) for the mitochondrial markers and Saint et al. (1998) for the nuclear one. Amplified fragments were sequenced on a 310 Applied Biosystem DNA sequencing apparatus.

Phylogenetic analyses

The sequences of the two mitochondrial regions (12s rRNA and 16s rRNA) were aligned in MAFFT (Katoh and Toh, 2008) using Q-INS-I algorithm, which takes secondary RNA structure into account. Following the methodology of Mason-Gamer and Kellogg (1996) there was no significant topological differences between estimates of phylogenies based on the individual mtDNA gene trees, and thus the two genes were analysed in a combined approach. A total of 920 bp (aligned) were obtained for mtDNA (373 for 12s rRNA and 546 for 16s rRNA). Including outgroups, a final alignment of 942 bp mtDNA combined data set (12s + 16s) was then analysed using maximum likelihood (ML) and Bayesian inference (BI) methods. The best-fit model of nucleotide substitution evolution under corrected Akaike Information Criterion was estimated using JModelTest 0.1.1 (Posada, 2008). Model TIM2 + G was chosen and

Table 1. *Ptyodactylus oudrii* list of samples, collection sites and GenBank accession codes. Coordinates are in decimal degrees, taken with a GPS (datum WGS84).

Code	Latitude	Longitude	Local	Region	Country	GenBank Accession Numbers		
						12s	16s	CMOS
906	35.225883	5.708083	El Kantara	Batna	Algeria	KC505506	–	KC583530
907	35.225883	5.708083	El Kantara	Batna	Algeria	KC505507	–	KC583531
908	35.225883	5.708083	El Kantara	Batna	Algeria	KC505508	–	KC583532
349	17.219600	8.099783	Agadez	Aïr	Niger	KC505509	KC505483	KC583520
350	17.219600	8.099783	Agadez	Aïr	Niger	KC505510	KC505484	KC583521
382	18.098383	8.766733	Timia	Aïr	Niger	KC505511	–	KC583522
383	18.098383	8.766733	Timia	Aïr	Niger	KC505512	–	KC583523
387	17.710817	8.274167	Elmiki	Aïr	Niger	KC505513	KC505485	KC583524
409	12.474800	2.427600	Tapoua	Niamey	Niger	KC505514	KC505486	KC583525
418	12.060333	0.369333	Fada N'Gourma	Gourma	Burkina Faso	KC505515	KC505487	KC583526
422	12.220017	0.301900	Fada N'Gourma	Gourma	Burkina Faso	KC505516	–	KC583527
429	12.353250	−1.527017	Ouagadougou	Kadiogo	Burkina Faso	KC505517	KC505488	KC583528
430	12.353250	−1.527017	Ouagadougou	Kadiogo	Burkina Faso	KC505518	KC505489	KC583529
3038	18.053485	−11.942891	Tin Waadine	Tagant	Mauritania	KC505519	KC505490	KC583533
3039	18.053485	−11.942891	Tin Waadine	Tagant	Mauritania	KC505520	KC505491	KC583534
3040	18.053485	−11.942891	Tin Waadine	Tagant	Mauritania	KC505521	KC505492	KC583535
3128	17.887298	−12.110844	Guelta Matmâta	Tagant	Mauritania	KC505522	KC505493	KC583536
3191	17.240833	−12.101667	Laout	Tagant	Mauritania	KC505523	KC505494	KC583537
3198	17.240833	−12.101667	Laout	Tagant	Mauritania	KC505524	KC505495	KC583538
3209	17.275708	−12.101020	Laout	Tagant	Mauritania	KC505525	KC505496	KC583539
3210	17.275708	−12.101020	Laout	Tagant	Mauritania	KC505526	KC505497	KC583540
3243	17.388097	−11.612028	Mendjoura	Tagant	Mauritania	KC505527	KC505498	KC583541
3317	16.876885	−11.963628	Touemief ed Demiâne	Assaba	Mauritania	KC505528	KC505499	KC583542
3369	17.070297	−12.207848	Oumm Icheglâne	Assaba	Mauritania	KC505529	KC505500	KC583543
3379	17.152482	−12.199115	Aouînet Nanâga	Assaba	Mauritania	KC505530	KC505501	KC583544
4798	15.957078	−12.009859	Guelta Goumbel	Guidimaka	Mauritania	KC505531	KC505502	–
4825	15.908323	−11.92121	Oued el 'Adam	Guidimaka	Mauritania	KC505532	KC505503	–
4847	16.547455	−12.00959	Foum Goussas	Assaba	Mauritania	KC505533	KC505504	–
4848	16.547455	−12.00959	Foum Goussas	Assaba	Mauritania	KC505534	KC505505	–

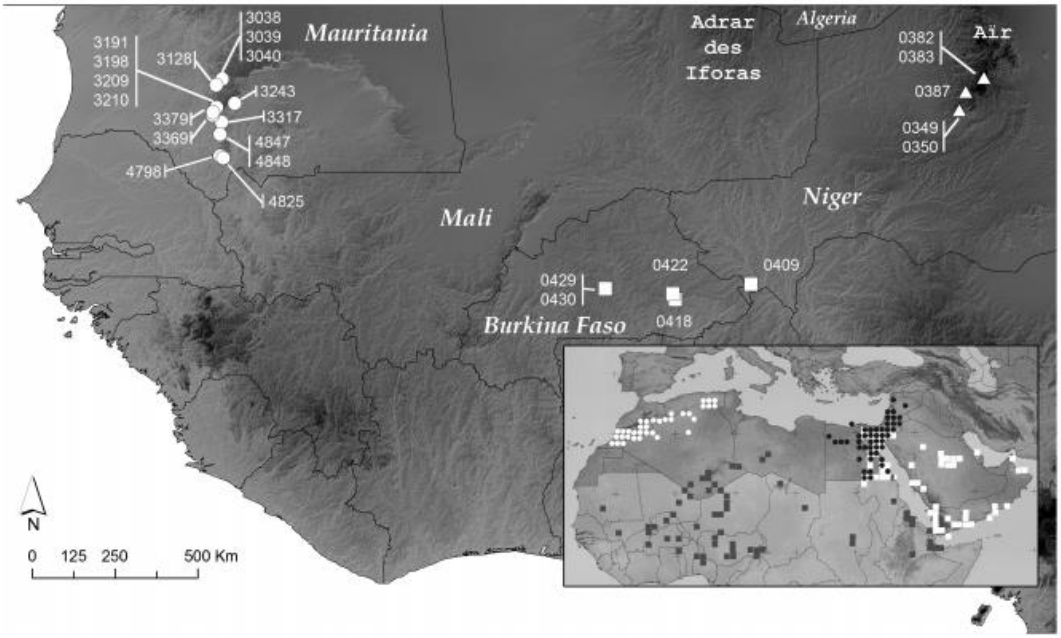

Figure 1. Study area and location of newly sequenced samples of *P. ragazzii*. Sample codes are indicated. Circles, triangles, and squares in the large map represent phylogenetic haplogroups obtained in the tree (fig. 2). Small map represents the distribution of the other species of *Ptyodactylus* included (modified from Sindaco and Jeremcenko, 2008 to include the recently identified Mauritanian population). White circles: *P. oudrii*; dark gray circles: *P. guttatus*; white squares: *P. hasselquistii*; dark gray squares: *P. ragazzii*.

used in the ML phylogenetic analyses. ML trees were built in PhyML (Guindon and Gascuel, 2003) with 1000 bootstrap replicates and searching for the best-scoring ML tree. Phylogenetic BI was performed on MrBayes version 3.1.2 (Ronquist, 2003). Sequences were partitioned according to genes. Each partition was allowed independent parameters of sequence evolution under the model GTR + I + G. Analyses started with program generated trees, with four heated Markov chains with default incremental heating; two independent runs 1.5×10^7 generations long were sampled at intervals of 500 generations producing a total of 30 000 trees. Burnin was determined upon convergence of log likelihood and parameter estimation values using Tracer 1.5 (Rambaut and Drummond, 2007).

Age estimations

In order to determine the approximate time scale of the splitting events among lineages, Beast 1.6.1 (Drummond and Rambaut, 2007) was used. Outgroups were removed from the sample set, since a coalescent approach was used. The dataset was run under a GTR + I + G substitution model, with substitution and clock models unlinked for 12s and 16s alignments. Given the unavailability of reliable calibration points for the genus *Ptyodactylus*, we used an average sequence evolution rate estimated for a closely related taxon (*Tarentola mauritanica*): 0.891 ± 0.00376% per million years for 12s rRNA (Carranza, S., unpublished data). An uncorrelated lognormal relaxed clock (Drummond et al., 2006) was chosen as the clock model for 12s, while 16s ran under a random local clock (Drummond and Suchard, 2010). The search comprised 1.5×10^8 generations, with tree sampling every 5000 generations. Quality of the runs was assessed and burn-in was determined using Tracer v1.5; 2500 trees were discarded. A maximum credibility tree with a confidence cut-off of 95% was then constructed using Tree Annotator (included in the BEAST package).

Genetic diversity

Regarding the nuclear region (Cmos), 339 bp were sequenced. In order to assess genetic variation, a haplotype network was produced using NETWORK 4.516 with a median-joining algorithm and default parameters (Fluxus Technology; Bandelt, Forster and Rohl, 1999). Heterozygotes were represented as independent haplotypes.

Estimates of evolutionary divergence over sequence pairs between groups and the average evolutionary divergence over sequence pairs within groups were calculated using MEGA5 (Tamura et al., 2011).

Results

A total of 63 samples of *Ptyodactylus* individuals were analysed for a final alignment of

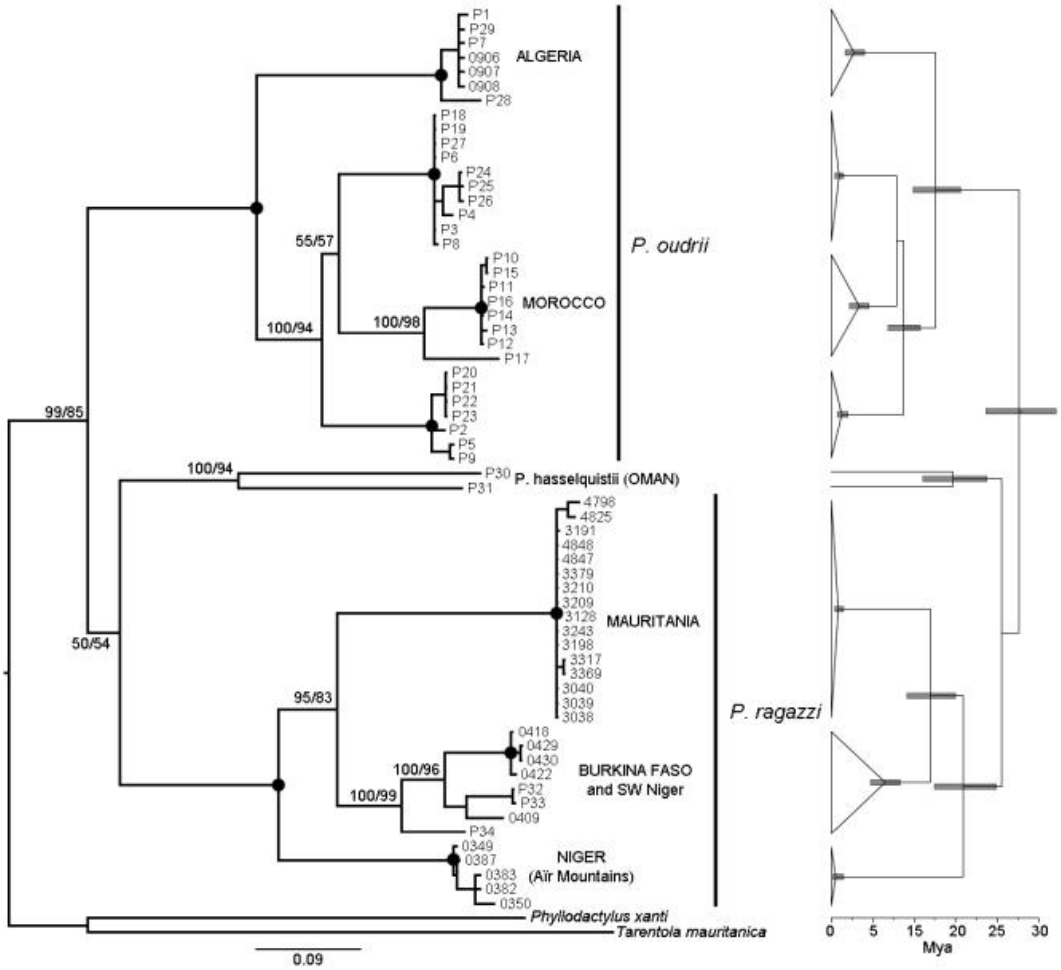

Figure 2. Left: Phylogenetic tree obtained by BI and ML analysis of the combined mtDNA fragments (12s and 16s). Support values (%) are given as Bayesian posterior probability/Bootstrap support. Dark circles indicate 100/100 support. Some terminal node values were omitted for clarity. Right: BEAST maximum clade credibility tree for the sampled *Ptyodactylus* lineages. Divergence times correspond to the mean posterior estimate of their age in million years. The grey bars indicate the height 95% HPD interval. Nodes with a posterior probability < 0.95 do not present a bar.

920 bp mtDNA combined data set (12s + 16s), of which 121 positions were variable and 101 parsimony-informative. A single tree was recovered from the ML analysis and the Bayesian analysis recovered the same tree as the ML analysis, with some minor incongruences in terminal topology, but statistically unsupported in either case (Bootstrap support < 70%; Bayesian posterior probability < 95%).

The results of the ML and Bayesian analyses of the mtDNA fragments are shown in fig. 2. As previously reported in Perera and Harris (2010), three highly supported clades were retrieved within *Ptyodactylus*, corresponding to *P. oudrii*, *P. ragazzii* and *P. hasselquistii*. The divergence between them is shown in table 2 (uncorrected distances for 12s rRNA), and varied from 17% between *P. oudrii* and *P. ragazzii* and 19% between *P. hasselquistii* and *P. ragazzii*.

Regarding *P. ragazzii*, three major mtDNA lineages were retrieved that are geographically concordant: one includes all the individuals from Mauritania; the second all the samples obtained from Burkina Faso and SW Niger,

Table 2. Estimates of Evolutionary Divergence over Sequence Pairs between and within Species; the number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal.

	P. ragazzii Niger	*P. ragazzii* Burkina Faso	*P. ragazzii* Mauritania	*P. oudrii*	*P. hasselquistii*
P. ragazzii Niger		0.011	0.017		
P. ragazzii Burkina Faso	0.065		0.015	0.016	0.017
P. ragazzii Mauritania	0.110	0.104			
P. oudrii		0.170			0.016
P. hasselquistii		0.189		0.175	

including previously published samples from Mali and Egypt (Perera and Harris, 2010), and basally a previously published sample from the Hoggar mountains in Algeria; and the third lineage includes the ones from the Aïr Mountains in Niger. The divergence between them is also shown in table 2. The maximum was retrieved between Mauritania and the Aïr Mountains in Niger with 11% (uncorrected distances for 12s rRNA) and the minimum value of 6.5% was observed between Mauritania and Burkina Faso and SW Niger. The analysis of the Cmos haplotypes is shown in fig. 3. All of the three lineages identified by mtDNA can be identified, with no haplotype sharing between them.

Finally, the three new individuals sequenced from *P. oudrii* (906, 907, 908; table 1) grouped together with previously *P. oudrii* individuals sequenced from Algeria (Perera and Harris, 2010) (fig. 2).

Age estimates for splits between the lineages indicate that they occurred in the Neogene, between 17 and 21 Mya (fig. 2).

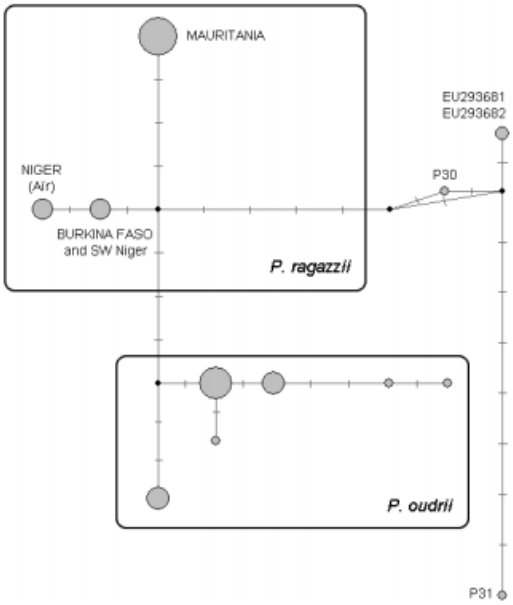

Figure 3. Haplotype (median joining) network showing the relationships of *Ptyodactylus* species, highlighted for *P. oudrii* and *P. ragazzii*, inferred from 339 bp Cmos nuclear sequences. Circle sizes are proportional to the number of samples sharing the same haplotype. Dashes represent mutated positions. Black circles represent median vectors.

Discussion

Our results have revealed the existence of three main allopatric and well supported (100% bootstrap and Bayesian posterior probabilities) monophyletic groups within *P. ragazzii* (fig. 2). The first group consists only of populations sampled from Mauritania. Previously known only from a single locality in the Tagant mountain (de la Riva and Padial, 2008), here we show that it is found over a larger area, from the Tagant in the north to the Assaba mountains 250 km to the south (fig. 1). Within the Mauritanian populations there is little variation (0.19% uncorrected distances for 12s rRNA), but the group is highly divergent (10% uncorrected distances for 12s rRNA; table 2) from its apparent closest relatives. This second clade comprises Sahelian samples (Burkina Faso and SW Niger), but also two individuals from Egypt and Mali (P32 and P33 respectively, fig. 2) and one from the Hoggar mountains Algeria (P34, fig. 2). Variation within this group is still high – the sample from the Hoggar mountains shows 5.3%

divergence from the rest of the group for example. Finally, the third clade includes the individuals sampled in the central-Saharan Aïr Mountains. All of these three clades also showed unique haplotypes for the nuclear marker Cmos, despite this being a slow evolving gene. Age estimates for splits between the lineages indicate that they occurred in the Neogene, between 17 and 21 Mya (fig. 2). While this is old relative to the estimate of the split between *P. guttatus* and *P. hasselquistii* (around 10 Mya, Gamble et al., 2011), it approximately coincides with separation of *Tarentola* species, but is young compared to the extremely deep time estimates for separation of species of *Saurodactylus* (nearly 100 Mya, Gamble et al., 2011). It is clear that many of the gecko species harbour very old lineages, but without more precise calibration points, estimates of separation dates remain quite speculative.

Although the Sahelian populations of *P. ragazzii* are quite widespread (see fig. 1), many of these populations, and particularly those from mountains in Mauritania, the Hoggar mountains and the Aïr mountains, are currently isolated by completely unsuitable habitat. Combined with the deep genetic divergences, at a level above proposed thresholds for candidate new species (Castiglia and Annesi, 2011), it seems that *P. ragazzii* as currently identified, is a complex of species. The type locality of *P. ragazzii* is in Eritrea (Anderson, 1898) and was not sampled in this study. Baha el Din (1999) suggests that morphologically Egyptian specimens are highly similar to the type series, thus indicating a possible link with Burkina Faso and SW Niger clade. However, given the deep divergences found within only a part of the known range in this study it is premature to make taxonomic changes without sampling the complete range, and without a detailed morphological assessment of these lineages.

Cryptic species seem to be common in North Africa herpetofauna, with many phylogeographic studies uncovering deeply divergent lineages that may correspond to new species (e.g. Barata, Carranza and Harris, 2012). Geckos in general seem to be a good example of this, as studies of *Tarentola* (Rato, Carranza and Harris, 2012) and *Quedenfeldtia* (Barata et al., 2012) indicated possible cryptic species. Other species such as *Saurodactylus brosseti* also present extremely high mtDNA variability (Rato and Harris, 2008), although the lack of clear geographical structure of genetic variation precludes conclusions about cryptic species. *Ptyodactylus oudrii* and now *P. ragazzii* are both apparently cryptic complexes, and probably *P. hasselquistii* from Oman also (fig. 2). This has important conservation implications, increasing the known biodiversity of this arid region but also reducing the ranges of species so that forms that were previously considered widespread now appear to be made up of many species currently known from only a few localities, and as such much more liable to extinction. For instance, populations of *P. ragazzii* in Mauritania were found exclusively in rock crevices surrounding rock pools (locally known as *guelta*), and are apparently limited by humidity. *Ptyodactylus* are naturally vulnerable to severe population extirpation or local extinction in areas suffering cyclic aridification. Although massive population reduction could be a part of these populations' history, conservation measures should be considered in order to perverse their uniqueness. Adding to the globally pressing concern of habitat loss and climate change, competition for water availability is particularly important in these areas. As an example, the relict fish fauna of the Adrar Mountains is highly endangered and several extinctions of fish populations have already occurred (Trape, 2009).

Saharo-Sahelian biodiversity possesses clear conservation and evolutionary interest, but only a few general publications present data about its distribution (Brito et al., 2011) and few studies are available on genetic variability, structuring and extinction risk (e.g. Froufe, Brito and Harris, 2009; Gonçalves et al., 2012). This study provides additional data on the biodiversity of

this poorly known region, and we hope will contribute to a better understanding of its history as well as to provide valuable information for conservation planning.

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Unravelling biodiversity, evolution and threats to conservation in the Sahara-Sahel

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ABSTRACT

Deserts and arid regions are generally perceived as bare and rather homogeneous areas of low diversity. The Sahara is the largest warm desert in the world and together with the arid Sahel displays high topographical and climatic heterogeneity, and has experienced recent and strong climatic oscillations that have greatly shifted biodiversity distribution and community composition. The large size, remoteness and long-term political instability of the Sahara-Sahel, have limited knowledge on its biodiversity. However, over the last decade, there have been an increasing number of published scientific studies based on modern geomatic and molecular tools, and broad sampling of taxa of these regions. This review tracks trends in knowledge about biodiversity patterns, processes and threats across the Sahara-Sahel, and anticipates needs for biodiversity research and conservation. Recent studies are changing completely the perception of regional biodiversity patterns. Instead of relatively low species diversity with distribution covering most of the region, studies now suggest a high rate of endemism and larger number of species, with much narrower and fragmented ranges, frequently limited to micro-hotspots of biodiversity. Molecular-based studies are also unravelling cryptic diversity associated with mountains, which together with recent distribution atlases, allows identifying integrative biogeographic patterns in biodiversity distribution. Mapping of multivariate environmental variation (at 1 km × 1 km resolution) of the region illustrates main biogeographical features of the Sahara-Sahel and supports recently hypothesised dispersal corridors and refugia. Micro-scale water-features present mostly in mountains have been associated with local biodiversity hotspots. However, the distribution of available data on vertebrates highlights current knowledge gaps that still apply to a large proportion of the Sahara-Sahel. Current research is providing insights into key evolutionary and ecological processes, including causes and timing of radiation and divergence for multiple taxa, and associating the onset of the Sahara with diversification processes for low-mobility vertebrates. Examples of phylogeographic patterns are showing the importance of allopatric speciation in the Sahara-Sahel, and this review presents a synthetic overview of the most commonly hypothesised diversification mechanisms. Studies are also stressing that biodiversity is threatened by increasing human activities in the region, including overhunting and natural resources prospection, and in the future by predicted global warming. A representation of areas of conflict, landmines, and natural resources extraction illustrates how human activities and regional insecurity are hampering biodiversity research and

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BIODIVERSITY RESEARCH

Conservation Biogeography of the Sahara-Sahel: additional protected areas are needed to secure unique biodiversity

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ABSTRACT

Aim Identification of priority conservation areas and evaluation of coverage of the current protected areas are urgently needed to halt the biodiversity loss. Identifying regions combining similar environmental traits (climate regions) and species assemblages (biogroups) is needed for conserving the biodiversity patterns and processes. We identify climate regions and biogroups and map species diversity across the Sahara-Sahel, a large geographical area that exhibits wide environmental heterogeneity and multiple species groups with distinct biogeographical affinities, and evaluate the coverage level of current network of protected areas for biodiversity conservation.

Location Sahara-Sahel, Africa.

Methods We use spatially explicit climate data with the principal component analysis and model-based clustering techniques to identify climate regions. We use distributions of 1147 terrestrial vertebrates (and of 125 Sahara-Sahel endemics) and apply distance clustering methods to identify biogroups for both species groups. We apply reserve selection algorithms targeting 17% of species distribution, climate regions and biogroups to identify priority areas and gap analysis to assess their representation within the current protected areas.

Results Seven climate regions were identified, mostly arranged as latitudinal belts. Concentrations of high species richness were found in the Sahel, but the central Sahara gathers most endemic and threatened species. Ten biogroups (five for endemics) were identified. A wide range of biogroups tend to overlap in specific climate regions. Identified priority areas are inadequately represented in protected areas, and six new top conservation areas are needed to achieve conservation targets.

Main conclusions Biodiversity distribution in Sahara-Sahel is spatially structured and apparently related to environmental variation. Although the majority of priority conservation areas are located outside the areas of intense human activities, many cross multiple political borders and require internationally coordinated efforts for implementation and management. Optimized biodiversity conservation solutions at regional scale are needed. Our work contradicts the general idea that deserts are uniform areas and provide options for the conservation of endangered species.

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